

STIMULATION OF MITOSES IN THE GERMINAL EPITHELIUM OF RAT OVARIES BY INTRACAPSULAR INJECTIONS¹

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THREE FIGURES

INTRODUCTION

The complete enclosure of the rat ovary by a membranous capsule provides a convenient space for the introduction of chemical substances whose direct effects on the germinal epithelium are to be tested. Estrone and thyroid substances so injected have been recorded as effecting mitotic stimulation (Stein and Allen, '42; Stein and Foreman, '49). In an attempt by the present writers to employ this technique for a study of the effects of nucleic acids and related substances, it became apparent that the method was complicated by injury responses, rendering it inherently unreliable. In the interest of recording the limitations of this procedure, which on first consideration appears so inviting, and to call attention to the extraordinary sensitivity of the germinal epithelium to mitotic stimulation, the following data are presented.

MATERIALS AND METHODS

All experiments were conducted on immature white rats of the Sprague-Dawley strain, which ranged in age from 21 to 52 days. Preparatory to injecting the periovarian space,

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the ovaries were retracted through dorsal incisions of the body wall, using ether anesthesia, and kept moistened with warm saline. The operation, including injection with a no. 27-gauge short-bevel hypodermic needle, was performed under magnification in an effort to prevent as far as possible any trauma through tearing of the delicate capsular tissue, puncturing of fine blood vessels, or scratching of the ovarian surface; specimens obviously injured were discarded.

The original intent was to inject nucleic acids and related substances. In order to prevent seepage of these chemicals out of the capsule it was thought desirable to suspend them in a coagulating medium, such as gelatin or agar. A 20% solution of Bacto gelatin was found to be sufficiently concentrated to set at body temperature and to remain fluid enough for passage through the hypodermic needle at 42°C. In the case of Bacto agar, a 1.4% solution met the same requirements. In consideration of the possible injurious consequences of a solidifying medium, isotonic saline was also used. The injection of any of these substances distended the periovarian capsule; gross ruptures seldom occurred, and any such specimens were discarded. Immediately after injection the ovaries were returned to the body cavity and the wound was sutured. With regard to the two ovaries of a pair, various combinations of treatment were made, so as to cross-check the effects of saline, gelatin, agar, and non-injection.

The ovaries were removed for examination after 24 hours. Ten hours previous to this time, a single subcutaneous injection of colchicine was made, in the dosage of 0.1 mg per 100 gm of body weight, so as to arrest the mitoses occurring in the germinal epithelium during the last 10 hours.

The excised ovaries, intact with capsule, were fixed in Zenker's fluid. Serial sectioning at 10 μ was followed by staining with iron hematoxylin. The mitoses in the germinal epithelium were counted in every 10th section. The germinal epithelium of these sections was then traced by projection and measured with a map measurer. The sum of the mitotic counts was divided by the sum of the epithelial measurements

after correction for magnification, giving for each ovary the number of mitoses per centimeter length of germinal epithelium.

RESULTS

The gelatin and agar injected under the periovarian capsules were retained after 24 hours, as shown in figure 1. In most instances neutrophiles were gathered at the edges of the coagula, indicating a traumatic or foreign-body response. Capsules filled with saline were collapsed at the time of excision, with no gross indication of the injected solution remaining. Colchicine-arrested mitoses were conspicuous in the germinal epithelium and the capsular lining of most injected specimens, but were relatively rare in the non-injected ovaries (cf. figs. 2 and 3). The mitotic rates obtained are given in table 1. Included in this table is also one set of data for injections of gelatin containing 0.1% of the enzyme ribonuclease. The mitoses stimulated by injection tended to be unevenly distributed over the surface of the ovary; they were not necessarily correlated with adjacent remnants of gel (no factor in the saline specimens) or associated with any particular region of the germinal epithelium.

Inspection of table 1 shows that the mitotic rate of the normal germinal epithelium (uninjected capsules) was fairly constant, with no significant variation over the age-range studied or between right and left ovaries. In the case of injected capsules, regardless of whether saline, gelatin, or agar was used, the mitotic rate was extremely variable and in most instances ran very high. Wherever one of a pair of ovaries was left uninjected, the injected ovary showed a many-fold increase in mitotic rate, which in one instance reached 56 times that of its mate. Frequently the injections raised the mitotic rate in the germinal epithelium 10 to 20 times above the normal range. There appeared to be no significant differences between the effects of saline, gelatin, or agar. The possibility that operative damage was greater in ovaries

with high mitotic rate than in the low ones could not be definitely excluded, but no clear indications of this were evident.

TABLE 1

Mitoses per centimeter length of germinal epithelium over 10-hr. colchicine period, following injection of periovarian capsule

OVARY NO.	AGE	UNIN- JECTED	SALINE	GELATIN	GELATIN AND RNASE	AGAR
	<i>days</i>					
D25a	21		2.44 L ¹			8.50 R ¹
D25b	21		5.45 L			6.26 R
D25c	21			7.60 L		5.40 R
C74a	21	10.49 L	42.80 R			
C74b	21		9.08 L			32.06 R
C74c	21		2.04 L			37.88 R
D22a	22	4.11 L	37.31 R			
D22b	22		11.78 L	15.32 R		
D21a	22	2.38 L	62.57 R			
D21b	22		17.10 L	83.02 R		
D13a	22		52.06 L			
D13b	22					43.69 R
D13c	22					11.92 L
D13c	22					56.24 R
D13d	22		18.95 L	36.91 R		
B109a	27	1.82 R			54.70 L	
C16a	43	2.55 R		54.43 L		
C16b	43			19.98 R		
C16b	43			27.54 L		
C16c	43			41.55 L	58.16 R	
C16d	43			17.06 R	24.79 L	
C47	52	3.14 R			177.40 L	
C46	52	2.68 R			44.68 L	
C51	52	2.13 R		37.39 L		
B104a	52			104.04 R		
B104a	52			80.76 L		

¹ L and R signify left and right ovary, respectively.

DISCUSSION

It is apparent from the results obtained that the technique of intracapsular injection, as here described, is not suitable for the testing of chemical substances with reference to their effects on mitosis in the germinal epithelium. Isotonic saline, agar, and possibly gelatin, can hardly be considered as any-

thing but inert so far as mitogenic chemical activity is concerned. The stimulating effects noted must be regarded as secondary and most likely result from some form of damage to the tissues bordering the site of injection. Sources of injury may include the penetration of the needle through the capsular wall; the distention of the capsule, producing small ruptures of tissue; abrasion by the coagulated agar or gelatin; and incidental derangements of membrane permeability. Such injuries may be inconspicuous but are inherently unpreventable. Evidences of mild inflammatory reactions were, in fact, not infrequently observed and included vascular dilation and leucocytosis. That the damaged tissues probably release growth-stimulating substances is a possibility supported by numerous studies on the properties of tissue extracts (Fischer, '46), the effects of radiation (Loofbourow, '48) and wound healing (Nutini, '50). Nucleoproteins and certain nucleic acid derivatives have been particularly suspected to be active in this capacity. The consequences of tissue damage with reference to cell proliferation are in any event such, that experiments dealing with the specific effects of chemical substances on cell division must be designed with the utmost caution.

The data of Stein and Foreman ('49) were of interest to the writers in the light of the present work. The technique employed by these investigators was similar to that reported here. It is evident that in their experiments Ringer's solution injected into the periovarian capsules (table 1, *op. cit.*) had the same mitosis stimulating effect found here for isotonic saline. Likewise, the range of variability was similarly high. Thyroid digests, injected into the capsules of mates to these ovaries, in most cases further raised the mitotic rates, but to highly varying degrees, and sometimes lowered them. The average number of mitoses was taken by Stein and Foreman to indicate mitotic stimulation as compared to the effects of Ringer injections. In view of the great variability of the mitotic counts this conclusion may be open to question. Their photomicrographs also show neutrophiles present in

the capsule, suggesting an inflammatory response. In the opinion of the present writers the amount of stimulation caused by the operation of injection is in itself so fortuitous that little certainty can be laid to the effects of added drugs.

SUMMARY AND CONCLUSIONS

1. Isotonic saline, gelatin, and agar were injected into the periovarian capsules of immature rats and their effects noted on mitotic rate in the germinal epithelium. Measurements were made for a 10-hour period following subcutaneous addition of colchicine.

2. The intracapsular injections elicited mitotic stimulation in varying degrees, most often very high, and in no apparent correlation with the type of substance injected.

3. The mitotic stimulation appears to be an injury reaction, as indicated by the peculiarities of the operational technique, the quantitatively variable results, and histological signs of inflammation.

4. The technique of intracapsular injection is considered unreliable for testing the effects of chemical substances on cellular proliferation in the germinal epithelium.

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PLATE

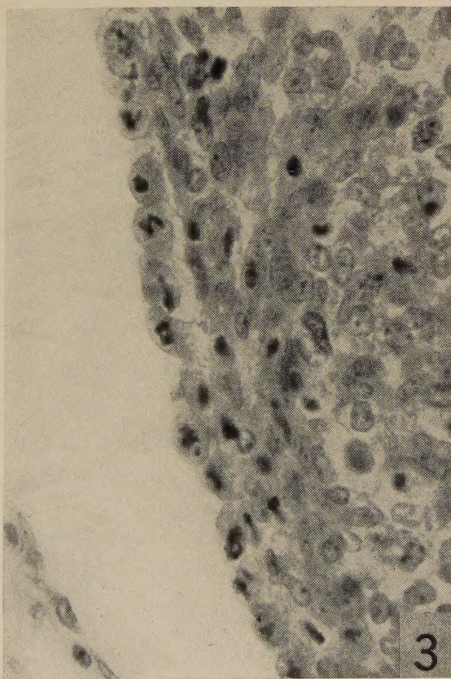
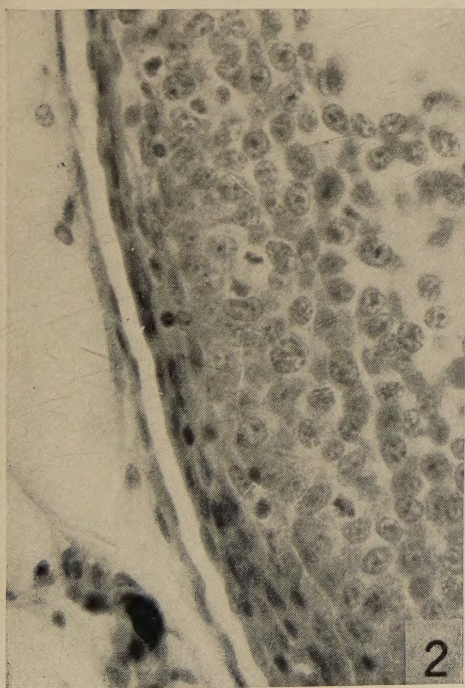
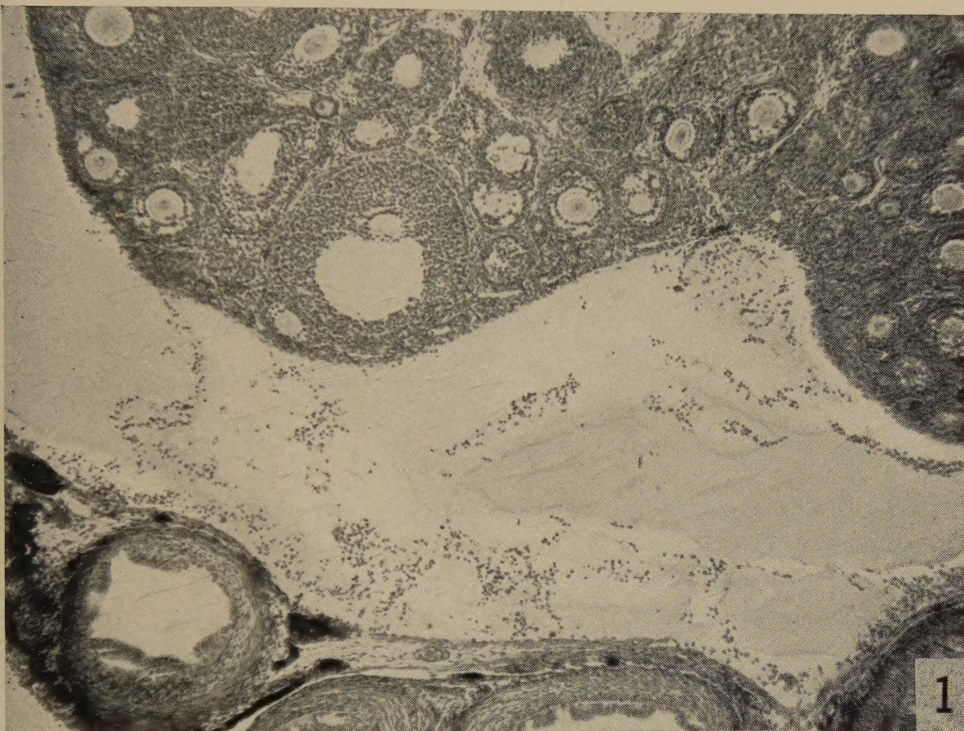
PLATE 1

EXPLANATION OF FIGURES

1 Ovary C74b-R, from 21-day rat, 24 hours after injection of agar into periovarian capsule. Note distention of periovarian space and neutrophiles gathered at edges of agar coagula. $\times 87$.

2 Ovary D21a-L, from 22-day rat. Periovarian capsule not injected, but colchicine administered subcutaneously 10 hours before removal. Compare appearance of germinal epithelium with that in figure 3. $\times 564$.

3 Ovary C47-L, from 52-day rat, 24 hours after injection of gelatin into periovarian capsule; colchicine administered subcutaneously 10 hours before removal. Note mitoses in germinal epithelium and compare with figure 2. $\times 564$.



EVALUATION OF HISTOCHEMICAL ALKALINE PHOSPHATASE TECHNIQS^{1,2}

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THREE FIGURES

The alkaline phosphatase technic of Gomori ('39) and Takamatsu ('39) has yielded valuable information on the nature of normal and pathological tissues. Successful use of the method for a precise localization of enzymatic activity depends on a good preservation of the enzyme during the preparation of tissues and the use of precautionary measures against loss or movement of substances in tissue sections. It is important, moreover, to realize certain limitations inherent in the technic and to interpret the results accordingly.

It has been pointed out recently (Lison, '48; Jacoby and Martin, '49) that the localization of enzyme activity may be partially obliterated by a non-specific precipitation resulting from diffusion of either the enzyme or reaction products from areas of high to low phosphatase activity. The last authors have produced evidence of a definite diffusion gradient from centers of high phosphatase activity and noted the presence of a particularly strong reaction in the nuclei in such areas.

Other critical papers considering the specificity of this technic have been published by Danielli ('46), Ruyter and Neumann ('49) and Gomori ('50b). Lorch ('47), in exam-

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ining the coupling azo dye technic of Menten, Junge, and Green ('44), concluded that it is inferior to that of Gomori and Takamatsu because of its failure to detect low concentrations of phosphatase in the nuclei. In the present study comparisons have been made between Gomori's technic and the azo dye technic of Menten, Junge, and Green ('44) as modified by Manheimer and Seligman ('48).

MATERIALS AND METHODS

A variety of tissues from normal strain A mice and Albany strain rats was fixed in several changes of chilled acetone for 24 hours. These were cleared in chloroform and infiltrated with two changes of 58 to 60°C. tissue mat for one hour. For the azo dye technic the fixation procedures recommended by Manheimer and Seligman ('48) were followed. All sections were cut at 6 μ and mounted on slides with acetone-water (1:1) on the warming plate at 37°C. The acetone-water was drained immediately after the tissues spread and the slides were dried for a few hours at room temperature. All blocks and sections were stored in the cold room at 7°C.

The sections were deparaffinized and incubated at 37°C. in Gomori's substrate mixture (Gomori, '43) at pH 9.4 for one and one-half hours routinely and for varying lengths of time in different experiments. Enzymatic activity was then visualized by the cobalt sulfide technic. No counterstain was used.

To observe any non-specific reactions, parallel control slides were prepared by one of three methods: (1) incubation in a control substrate mixture without the glycerophosphate; (2) incubation in a standard substrate containing 0.01 M KCN as a specific inhibitor of the enzyme; and (3) use of inactivated slides in a standard substrate. The enzyme was destroyed by three methods of inactivation: (1) Lugol inactivation; after 15 minutes in Lugol's solution followed by 5 minutes in 2.5% sodium thiosulfate, slides were washed in running tap water for 30 minutes. (2) 1 N HCl inactivation; sections, after 15 minutes in 1 N HCl, were washed in running tap water for 30 minutes. (3) Hot water inactivation; slides immersed in

water were heated gradually to 90 to 100°C. for 10 to 15 minutes. Distortion in cytological details was avoided by increasing the temperature gradually. The degree of inactivation was checked in each experiment by incubating these slides in a standard substrate.

For the coupling azo dye technic of Manheimer and Seligman the slides were deparaffinized and incubated for 20 minutes to 1 hour at 7°C. in a freshly prepared substrate mixture. All ingredients were kept and mixed in the cold room. The calcium beta naphthyl phosphate and alpha naphthyl diazonium naphthalene-1, 5-disulfonate synthesized in this laboratory were compared with those obtained from Monomer-Polymer, Inc., 3430 West Henderson Street, Chicago 18, Illinois. Similar results were obtained with both samples.

After incubation the slides were washed in running tap water for 5 minutes and mounted with Kaiser's glycerol gelatin (Mallory, '38, p. 100). Some slides were counterstained with dilute hematoxylin. Two types of controls were used: (1) inactivated slides incubated in the normal substrate and (2) normal slides incubated in a substrate mixture lacking the calcium beta naphthyl phosphate.

EXPERIMENTAL PROCEDURES AND RESULTS

A. Diffusion of substances in Gomori's technic

A certain amount of extraction of the water soluble alkaline phosphatase may be expected with incubation of tissues at 37°C. in a substrate at pH 9.4, since extraction of proteins and other substances from tissues occurs readily with increased alkalinity. Several experiments were designed to examine critically the possibility of diffusion and adsorption of the enzyme itself and the reaction products in Gomori's technic. Tissues from normal mice, unless otherwise stated, were used in the following experiments.

1. Incubation of tissues for varying lengths of time showed a progressive spread of the phosphatase reaction from centers of high activity to surrounding areas. This was particu-

larly noticeable around the strongly active small blood vessels in the liver, pancreas, and salivary glands. The reaction occurring in cells adjacent to the blood vessels was conspicuous in the nuclei and less so in the cytoplasm.

Rat testis incubated for 5, 10, 15, 60 and 90 minutes showed a similar spreading of activity from the strongly staining basement membrane of the seminiferous tubule to the developing sperm cells. This appeared to be due, in part, to a diffusion of either the enzyme itself or the reaction product from the basement membrane.

At 5 minutes the endothelium of small arteries was the only structure stained in the testis. At 10 minutes a pale, spotty reaction appeared in the basement membrane of the tubules and a light brown staining occurred in the large nucleoli of Sertoli cells.

The results at the end of one and one-half hours were most informative (fig. 3). Occasionally the basement membrane was separated from cells due to artifact. In regions where the cells remained in direct contact with the strongly reacting basement membrane, the nuclei of the spermatogonia and primary spermatocytes were heavily stained. On the other hand, where the basement membrane was separated from the adjacent layer of spermatogonia and Sertoli cells, there was no reaction in these cells except for a weak nucleolar staining in the latter. Less reaction occurred in the cells with increased distance between the membrane and the cells. It is evident from these findings that cellular nuclei have a special affinity for the diffusing substance involved in this reaction.

2. The diffusion of substances from areas of high to low enzyme activity can be demonstrated experimentally. By superimposing a tissue rich in alkaline phosphatase over an inactivated section and incubating this preparation (Danielli, '46), a definite reaction is obtained in the subjacent tissue.

Liver sections inactivated by hot water, Lugol's solution, and 1 N HCl were partially covered with fresh paraffin sections of kidney. For inactivation with 1 N HCl and Lugol's

solution, non-deparaffined sections yielded better cytological detail in this procedure. After deparaffinizing, these preparations, with and without celloidin coating, were incubated in standard substrate for one and one-half and three hours.

The results showed a marked, consistent diffusion gradient from the overlying renal cortex to the underlying inactivated liver for a distance of approximately 100 μ . There was a selective adsorption of the diffusing substances in the liver nuclei, chiefly in the chromatin and nucleoli, as evidenced by a particularly strong reaction in these structures and a weak staining in the cytoplasm. The amount and extent of diffusion was greater in those slides without the celloidin coating.

Similar results were obtained when bisected duodenum sections were used as the overlying material. These preparations were incubated for 3, 24, 48 and 72 hours. With prolonged incubation the amount and extent of diffusion increased.

The cytological localization of the adsorbed substance in the subjacent tissue differed with the three methods of inactivation. In sections treated with 1N HCl there was a pronounced nuclear and slight cytoplasmic reaction. Those inactivated with Lugol's solution generally gave a dark nuclear and cytoplasmic reaction. After hot water inactivation the general result was weaker than with either of the other two methods.

3. Evidence was obtained from the following experiment that it is primarily the enzyme which diffuses to neighboring structures during incubation. A fresh paraffin ribbon of duodenum was pre-incubated in the standard substrate for one-half, one and one-half and three hours, thus forming a rich deposit of calcium phosphate while retaining some of its enzymatic activity. The sections were washed in distilled water for 5 minutes, bisected, and mounted on inactivated liver sections by the method described above.

The superimposed preparations were incubated for 3 and 5 hours in (a) standard substrate and (b) substrate containing 0.01 M KCN, inhibitor for intestinal alkaline phosphatase.

Normal duodenum slides incubated in substrate (b) were used as controls to check the degree of inhibition by 0.01 M-KCN since at times this inhibition may not be complete (Gomori, '49a).

Diffusion from the overlying to the underlying tissue occurred in all superimposed preparations incubated in the standard substrate (fig. 1). This diffusion was not visible in slides from substrate (b) containing the enzymatic inhibitor. In all instances the KCN completely inhibited the activity in control duodenum sections. Inactivated controls were negative.

In this study the histochemically detectable diffusion to subjacent cells was primarily that of the enzyme itself and not of the calcium phosphate during and subsequent to incubation. This conclusion is derived from the fact that the diffusion picture was present only after incubation in the standard substrate. A negative reaction of subjacent cells in the presence of KCN indicates that the activity of the adsorbed enzyme was inhibited. If there had also been a detectable movement of the calcium phosphate, a positive reaction should have resulted even after the KCN treatment. Whether there was any loss of calcium phosphate into the substrate mixture could not be determined.

4. Additional evidence of extraction of the enzyme from tissue sections was gained from slides pre-incubated in the control substrate. Duodenum sections, with and without celloidin coating, were immersed in the control substrate lacking glycerophosphate at 37°C. for one and one-half and three hours. For comparison some slides were placed in distilled water and otherwise treated similarly. All of them were then incubated in the standard substrate for one and one-half hours along with untreated normal sections.

Pre-incubation in the control substrate caused a visible reduction in activity but the distilled water produced no change. A celloidin coating afforded only a partial protection, for diffusion occurred to the extent of several microns along the striated border. This leads one to believe that at pH 9.4

under conditions similar to that of the normal substrate except for the absence of glycerophosphate, a certain amount of the duodenal phosphatase is extracted. It is probable then, that with incubation in the standard substrate there is a similar loss of enzyme from the duodenum, although perhaps not to the same degree.

5. Inasmuch as the above experiments indicated a diffusion of the enzyme and a selective adsorption by the nuclei, it was thought desirable to study the adsorption of purified phosphatase by inactivated tissue sections.

Enzyme solutions were prepared from defatted, dehydrated hog kidney powder obtained from the Viobin Corporation, Monticello, Illinois. Fifty grams of the powder were placed in the Waring blender with 250 cm³ of distilled water for 5 minutes, centrifuged, and the extract was dialyzed against distilled water for 2 to 4 days. A white precipitate which formed during dialysis was removed by centrifugation. Alkaline phosphatase activity was determined chemically by a method devised in this laboratory using disodium phenylphosphate as substrate. (Observations to be published.)

Small quantities of enzyme were placed in glass rings over a variety of inactivated tissues and evaporation was prevented by cover-slips. These slides were kept at 37°C. for 1½, 3 and 5 hours and subsequently either rinsed briefly or washed in running tap water for 30 minutes. Following this they were incubated in the standard substrate for one and one-half hours and the activity visualized by the cobalt sulfide method. Some slides were incubated in a control substrate lacking the glycerophosphate.

The results were strikingly similar to the reaction obtained in the underlying inactivated livers in earlier experiments (fig. 2). The cobalt sulfide occurred predominantly in the nuclei, especially in the chromatin and nucleoli. A light brown reaction was present in the cytoplasm. Washing for one-half hour in running tap water before incubation in the substrate removed some of the general background staining, but the strong selective reaction in the nuclei and a pale brown

color in the cytoplasm persisted. Slides from the control substrate gave negative results, which would eliminate the possibility of non-specific precipitation from the enzyme solution if it had contained any impurities, and would indicate that the positive cobalt sulfide reaction was due entirely to enzymatic activity. The inactivated controls were negative.

6. Since a flocculent white precipitate of calcium phosphate forms in the standard substrate with prolonged incubation, the additional problem of its adsorption by tissues was investigated.

The affinity of tissues for calcium phosphate was determined by causing a hydrolysis of the substrate with the addition of commercial 3% hydrogen peroxide (Danielli, '46). This produced a slow and constant precipitation of calcium phosphate in the substrate mixture.

After inactivation by hot water, sections from a variety of tissues were incubated in standard substrates containing 1 cm³ and 5 cm³ of hydrogen peroxide per 100 cm³ of solution. Slides were incubated for one-half, one and one and one-half hours. Following incubation some of the slides were washed in running tap water for 15 minutes and the deposited calcium phosphate was visualized by the usual procedure. Quantitative chemical data were taken for the amounts of calcium phosphate present in the substrates at different times.

The results obtained from this experiment are summarized in table 1. A slight general staining occurred at one-half hour incubation. The nucleoli and chromatin in all tissues were fairly prominent and the cytoplasm pale brown after one hour. No significant increase in staining occurred after one and one-half hours. Results from the substrate containing 5 cm³ of hydrogen peroxide were not perceptibly darker than from the weaker solution, although chemical analyses showed that considerably more calcium phosphate was being precipitated in the first solution. Washing slides in running tap water reduced the cytoplasmic reaction but left the nuclear activity unaltered.

Although Danielli ('46) stated that the precipitation of calcium phosphate occurs at those sites showing true alkaline phosphatase activity, the results obtained here are not in complete agreement. Neither the blood vessels nor the brush border of renal tubules, which normally contain considerable amounts of alkaline phosphatase, show a special affinity for free calcium phosphate as do the nuclei. All nuclei in a variety of inactivated tissues adsorb the precipitating calcium phosphate.

TABLE 1

Determinations of calcium phosphate in substrates containing hydrogen peroxide

INCUBATION TIME IN HRS.	1 CM ³ H ₂ O ₂ PER 100 CM ³ SOLUTION			5 CM ³ H ₂ O ₂ PER 100 CM ³ SOLUTION		
	μg P liberated per cm ²	pH	Histo- chemistry	μg P liberated per cm ²	pH	Histo- chemistry
$\frac{1}{2}$	.609	9.0	0-1	2.91	8.9	0-1
1	.878	..	2	5.77	..	2
1 $\frac{1}{2}$	1.55	8.9	2	7.21	8.9	2 +

Phosphorus determinations were made by the Fiske-Subbarow method.

Histochemical results:

0-1 Slight general background staining over the control inactivated slide.

1 Darkening of nucleoli and chromatin.

2 Much darker nucleoli and chromatin than 1.

2 + Slightly darker reaction than 2.

In our experience the substrate does not ordinarily contain significant amounts of calcium phosphate as determined chemically after short incubation periods. With prolonged periods such as 72 hours there is a possibility of sufficient quantities of calcium phosphate being formed to cause an artifact.

7. Another experiment was designed to study the effects of diffusing and dissolving calcium phosphate on normal tissues. Sections of duodenum were pre-incubated in a standard substrate, rinsed, and partially covered with normal liver. These slides were then incubated in the control mixture lacking the glycerophosphate. A certain amount of calcium phos-

phate was expected to dissolve into the solution. At the end of three hours the calcium phosphate in the tissues was visualized by the usual technic. There was only a pale yellow reaction in the nuclei of the overlying liver which would ordinarily be considered as a negative reaction. This indicates that the amount of diffusion is so small that it is negligible for the time employed. This and other experiments in which the control substrate was saturated with calcium phosphate show that in the dissolved state it does not adhere to the nuclei.

8. The significant role of the common ion in reducing the solubility of calcium phosphate can be demonstrated by simple experiments. Calcium orthophosphate is soluble to the extent of 2-3 mg per 100 cm³ of water at 20°C., a quantity which requires consideration in histochemistry. However, under our experimental conditions, with an excess of calcium ions at an alkaline pH, the solubility is extremely low due to the common ion effect. Chemical determinations of soluble calcium phosphate in the presence and absence of calcium ions in the standard Gomori substrate disclosed that the solubility of calcium phosphate is reduced by approximately 5 times with the prescribed amount of calcium.

The marked difference in the solubility of calcium phosphate under various conditions was studied histochemically. Slides of rat testis were incubated for 15 minutes in the standard substrate to precipitate the calcium phosphate in the basement membrane and then immersed for one and one-half hours at 37°C. in (a) distilled water, (b) barbital buffer at pH 9.4, (c) substrate control, and (d) substrate containing 0.01 M KCN, an inhibitor for the phosphatase in the basement membrane of seminiferous tubules. For controls the calcium phosphate was visualized by cobalt sulfide after the 15 minutes incubation.

There was no cobalt sulfide precipitation in the basement membrane of slides immersed in (a) distilled water and (b) barbital buffer. Those in the substrate control solution and the substrate containing KCN showed a good reaction com-

parable to that of controls. The latter gave a marked reaction in the basement membrane and a slight reaction in cells adjacent to it.

The loss of calcium phosphate in the slides from (a) and (b) may be explained on the basis of a lack of the common ion effect. In the absence of the common ion, calcium, the extremely small quantity precipitated in the tissue section was found to dissolve in solutions (a) and (b). In its presence (solution c and d) the precipitate was retained. That the slightly acid pH of the distilled water was not a factor in the loss of calcium phosphate in (a) can be readily seen since solution (b) consisted of barbital buffer at pH 9.4.

Similar results were obtained when calcium phosphate was precipitated in the duodenum and this was superimposed on inactivated liver. After three hours of incubation there was little or no precipitate remaining in the slides from (a) and (b). There was only a slight loss in (c). It is of significance that despite the loss of calcium phosphate from the overlying duodenum in (c), there was no evidence of its deposition in the underlying tissue.

The common ion effect aids in preserving the calcium phosphate deposited in the tissues after the usual incubation procedure. The calcium nitrate rinse recommended by Gomori ('41) should be utilized. A rapid rinse in distilled water will not affect the results but a wash of more than a few minutes will cause a visible reduction in the precipitate.

B. Observations on Manheimer and Seligman's azo dye technic

A variety of mouse tissues was stained by the azo dye technic and compared with those by Gomori's method. A brilliant purplish-red staining was obtained by the azo dye method in tissues containing high enzyme concentrations, as in the renal cortex, intestinal mucosa, and small blood vessels. The dye deposited in the form of a homogeneous mass in areas rich in phosphatase and as scattered, coarse granules

where the activity was weaker. Under the conditions employed, unfixed frozen sections did not give satisfactory results with the Manheimer-Seligman technic because of the crystalline dye precipitate.

Noticeable differences were observed in the results obtained by the two technics particularly in the liver. By Gomori's method activity was found in the branches of the hepatic artery, portal vein, bile duct, bile canaliculi and sinusoids. Liver cell nuclei stained darkly in the portal areas. With the azo technic only branches of the hepatic artery and sometimes the portal vein were stained. There was a conspicuous lack of staining of nuclei and most bile canaliculi in the normal liver.

There was generally a negative reaction in the cell nuclei with the azo technic whereas with Gomori's test a dark nuclear reaction occurred in many tissues. Inconstant results were obtained in this respect with the new method in the duodenum and renal cortex where sometimes the nuclei contained heavy deposits of dark red dye. This was particularly true after 95% alcohol fixation. Some of this nuclear reaction in the azo dye technic was found to be an artifact. When tissues fixed in 95% alcohol were stained and mounted, the nuclei in areas of high phosphatase concentration exhibited enhanced dye deposition upon standing at room temperature for several days to a few weeks. This process was hastened by heating in the oven at 60°C. for a few hours. The non-enzymatic nature of this reaction was established when the dye continued to accumulate in the nuclei after the following treatment. Sections were incubated, washed in running tap water for 5 minutes, and then inactivated by 1 N HCl and Lugol's solution. The dye was insoluble in these solutions. Since the accumulation of dye continued after destruction of the enzyme, this deposition must be attributed to a non-specific reaction.

The negative reaction of the bile canaliculi and the nuclei in tissues other than kidney and duodenum was not due to the slow rate of enzymatic reaction at 7°C. as contrasted with

37°C. in Gomori's technic. Extended incubation times up to 7 hours with hourly changes of the substrate showed no further appearance of the dye in structures not stained at one hour. There was a slight progressive increase in staining intensity in the first few hours in structures already stained at 20 minutes to 1 hour. With Gomori's substrate it required 5 hours of incubation at 7°C. to obtain results equivalent to those after one and one-half hours at 37°C.

Because of the discrepancy in the bile capillary and nuclear reaction with the two technics, it was suspected that the azo dye method was perhaps less sensitive than that of Gomori's. The two methods were therefore compared with respect to (a) regenerating liver after partial hepatectomy which contains increased amounts of alkaline phosphatase (Sulkin and Gardner, '48; Brachet and Jeener, '48; unpublished data from this laboratory) and (b) enzyme-gelatin preparations of known concentrations.

Regenerating livers were obtained from 18 3-months old male mice. Two-thirds of the liver, the left lateral and the median lobes, were removed under ether anesthesia and the regenerated portions excised three days later. The animals were unstarved throughout the experiment.

This rapidly growing liver tissue showed an intense bile capillary and nuclear reaction after Gomori's test. With the azo dye technic the bile capillaries stained prominently but the nuclei were still negative. This provided evidence that with this method the dye is not deposited until a certain concentration of enzyme is exceeded. This amount is probably considerably more than that required to produce a visible precipitate in Gomori's technic. The possibility cannot be excluded, however, that the azo dye method demonstrates enzymes other than that found with the Gomori method.

The reaction of these two technics was compared by utilizing enzyme extracts of known concentrations on slides. The average activity of stock enzyme samples by chemical determinations was 5.0 mg of phosphorus per cubic centimeter of solution released from disodium phenylphosphate per hour

at pH 9.2. Enzyme solutions were diluted with 1% gelatin and the various dilutions were written with a steel pen on slides previously coated with albumen (Gomori, '50a). After brief fixation in chilled acetone or alcohol, they were tested by the two technics.

With extracted enzyme mixtures both Gomori's and Manheimer and Seligman's technics were equally sensitive to one-twentieth of the original concentration, while in lower concentration the detection of activity was doubtful. This finding indicated that the lack of reaction in some cytological structures in tissue sections with the azo dye technic may perhaps be due to physico-chemical limitations.

Because of the conspicuous difference in the alkaline phosphatase activity of liver nuclei with the two technics, an attempt was made to correlate the histochemical results with quantitative chemical determinations on isolated nuclear fractions. The chemical studies yielded no convincing evidence for an obvious concentration of activity in isolated liver nuclei.

DISCUSSION

The alkaline phosphatase method has been considered the most reliable of the histochemical enzyme technics devised by Gomori. Danielli ('46), after an extensive study on various aspects of the technic, reported that it was satisfactory for localization of the enzyme. Our results show that with proper precautions, this method is a sensitive tool for the identification of alkaline phosphatase activity in tissue sections.

The present studies confirm the observations of Lison ('48) and Jacoby and Martin ('49), however, that there is a complicating factor of enzyme diffusion in Gomori's technic. Moreover, it has been shown that nuclei of all tissues have a special affinity for the enzyme. This fact, however, does not detract significantly from the specificity of the technic. It necessitates the use of different means of reducing enzyme diffusion and more careful observations and interpretation of results. Neither does this denote a complete absence of alkaline phos-

phatase activity in the nuclei. Willmer ('42) and Krugelis ('42) reported its presence in chromosomes histochemically and Danielli and Catcheside ('45) observed its distribution in chromosomes at the Feulgen positive sites. Using partially depolymerized desoxyribonucleic acid, Krugelis ('46) obtained strong reactions in nuclei and chromosomes. Mirsky ('47) found this activity in the residual chromosome of the lymphocyte. Furthermore, Brachet and Jeener ('48) cited examples of darker reactions in the nuclei of regenerating liver and other tissues where rapid growth is associated with protein synthesis. Ruyter and Neumann ('49), on the other hand, considered the nuclear deposition as an artifact on the basis of positive reactions in their control sections.

Although not conclusive, our quantitative chemical analyses indicate that in mouse liver the alkaline phosphatase activity is not concentrated in the nuclear fraction. On the contrary, in rat liver Dounce ('43) has reported a high concentration of the enzyme in the nuclei. Although the chemical data from fractionation studies aid in the localization of substances in cells, it should be remembered that the technics now available are beset by certain limitations. By disrupting the organization of tissues and cells and repeated washing, it is likely that various chemical systems are disturbed or destroyed. Some of these substances may be extracted and adsorbed by structures having a special affinity for them. It is difficult to estimate the extent of such processes.

The large initial loss of enzyme activity in the preparation of tissues for histochemistry should be borne in mind (Stafford and Atkinson, '48; Doyle, '48). Lison stated that a positive reaction may denote the presence of the enzyme but that a negative reaction does not indicate its absence. Other sources of error in enzyme histochemistry have been discussed by Gomori ('49a and '50b).

Under ordinary conditions of incubation the solubility of calcium phosphate is considerably reduced owing to the common ion effect produced by an excess of calcium ions. In all probability, the production rate of phosphate ions due to

enzymatic activity soon overtakes that of diffusion, so that the solubility product of the calcium phosphate is exceeded locally and therefore precipitated. Our studies show that once precipitated, the amount of calcium phosphate which diffuses to neighboring structures during short incubation periods at pH 9.4 is so small as to be negligible. It is undesirable to wash the sections for great lengths of time in distilled water after incubation, for this dissolves some of the calcium phosphate. The recommended calcium nitrate rinse should be utilized.

Gomori ('50b) demonstrated that with prolonged incubation artifacts arise from supersaturation of the incubating medium with hydrolysis products. He observed this to be true with pH values below 8.5 but not at 9.2. Danielli ('46) found that calcium phosphate is adsorbed by inactivated tissues from supersaturated incubating mixtures produced by addition of hydrogen peroxide. Our results show that such adsorption occurs particularly in the nuclei of various tissues.

By using superimposed preparations Danielli ('46) concluded that calcium phosphate formed in areas of high phosphatase activity does not diffuse in significant amounts to areas having a special affinity for it. With a modification of his technic our experiments have shown that although the calcium phosphate does not move in appreciable amounts, there is a definite diffusion of the enzyme itself which may give a false positive reaction.

The azo dye method while being as sensitive as the Gomori technic when tested with extracted enzyme preparations, fails to detect low concentrations in tissue preparations. Because of the large size of the substrate molecules, the reaction may be hindered by physico-chemical difficulties. Although this reaction occurs rapidly and reaches a maximum at one hour, the technic has its limitations in cytological use for three reasons: (1) it fails to detect low concentrations of enzyme in tissue sections; (2) it forms a coarse granular precipitation instead of a more desirable homogeneous deposition

where the enzyme is present in moderate amounts; and (3) it produces some artifacts in the nuclei.

Some of the advantages of this technic for histological studies are: (1) the ease in studying calcified material since the dye can be deposited in the tissue block first and then decalcified before sectioning by the freezing method and (2) its rapidity for survey work involving tissues with high alkaline phosphatase. For the reasons mentioned above this azo dye technic as employed can be used profitably for histological studies but not for detailed cytological examinations.

SUMMARY

1. Gomori's alkaline phosphatase technic has been compared with the azo dye method of Manheimer and Seligman.

2. When tested with extracted alkaline phosphatase-gelatin mixtures both technics give a similar reaction. The azo dye method, however, requires a higher concentration of the enzyme in tissue sections than the Gomori method before detection is possible.

3. Caution should be taken in the interpretations of results in Gomori's technic, since there is evidence of diffusion of the enzyme and its selective adsorption by nuclei. Precautions and proper control studies are essential.

4. The nuclei will adsorb calcium phosphate also when it is present in supersaturated amounts in the substrate.

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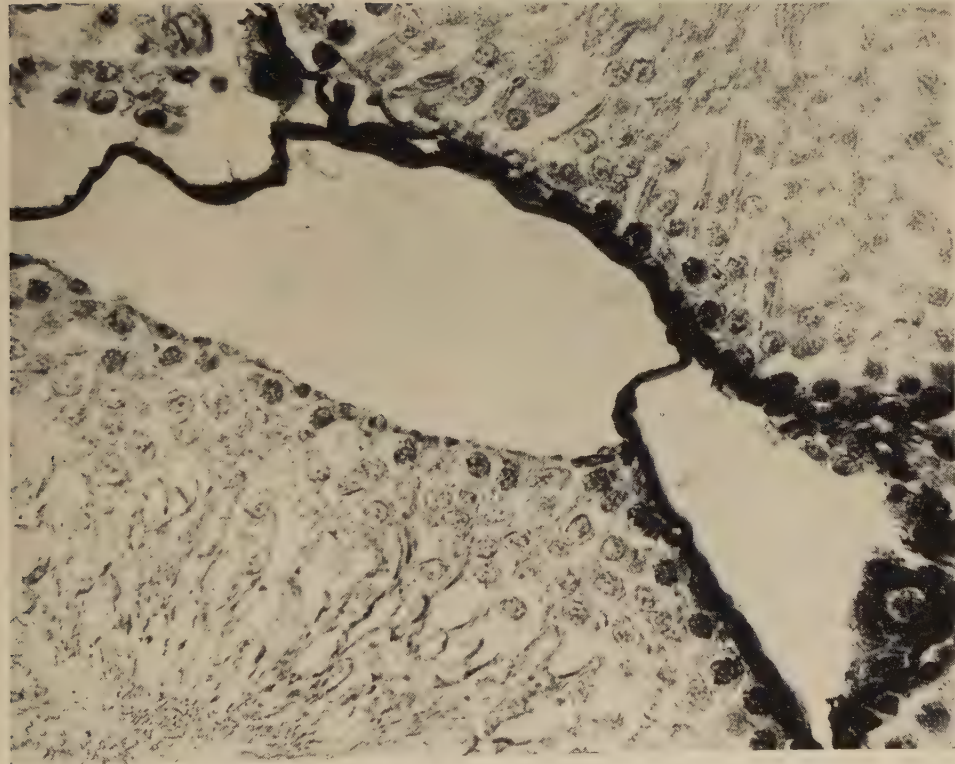
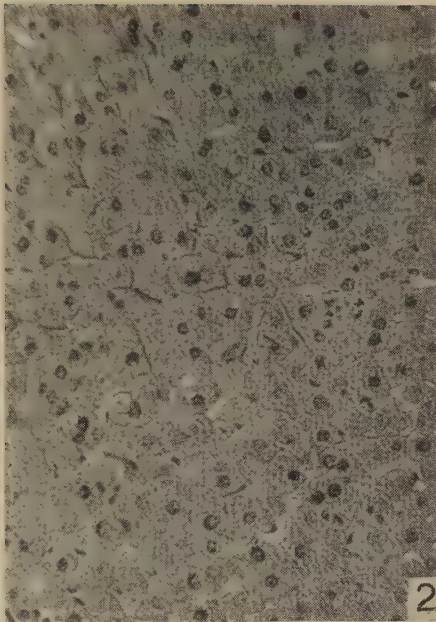
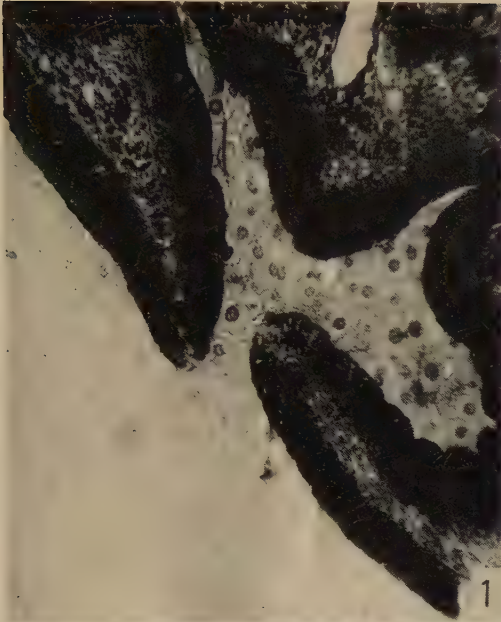
PLATE 1

EXPLANATION OF FIGURES

1 Superimposed preparation of mouse tissues showing a diffusion gradient of alkaline phosphatase activity after three hours of incubation in Gomori's substrate. The overlying duodenum was pre-incubated for one and one-half hours in the glycerophosphate substrate and therefore contained a rich deposit of calcium phosphate while retaining some of its enzymatic activity. The subjacent liver tissue was inactivated. This diffusion picture was absent in similar superimposed preparations incubated in a substrate containing 0.01 M KCN. $\times 200$.

2 Inactivated mouse liver showing a strong selective nuclear adsorption of alkaline phosphatase solution which had been extracted from hog kidney powder. Gomori's technic. $\times 200$.

3 Testis of rat after one and one-half hours incubation, showing a diffusion of alkaline phosphatase from the basement membrane to adjacent cells. The spermatogonia in close contact with the basement membrane are stained heavily. There is less reaction with greater separation from the basement membrane. Gomori's technic. $\times 500$.



A MICROSCOPIC STUDY OF THE ADRENAL OF THE BROWN PELICAN¹

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SIXTEEN FIGURES

INTRODUCTION

The results of previous work on the adrenal have directed our attention to the wide range of variation that occurs, particularly in the cortical or interrenal component, among members of different vertebrate groups. These variations involve differences in adrenal-body weight ratios, in relative amounts of interrenal and chromaffin components, in histological and cytological structure and in lipid and cholesterol content. Certain combinations of features appear distinctive for classes, orders, families and even for species of the same genus; and within species there are, in some cases, sex differences.

The significance of these differences is problematical but present knowledge of the histophysiology of the adrenal cortex suggests that they reflect functional variations. It has been demonstrated experimentally in the rat and guinea pig that increased activity of cortical cells is indicated by a fall in cholesterol and vitamin C content and decreased activity by an accumulation of these substances, effects that are known to be regulated by variations in the output of adrenocorticotrophic hormone (Sayers et al., '46). There is suggestive evidence that different hormones are produced by histologically different regions of the cortex in the rat. Deane and Greep ('46) concluded that the sodium hormone is produced in

¹ Aided by a grant from The Comly Fund of The Ohio State University.

the zona glomerulosa unregulated by the pituitary, whereas the gluconeogenic hormone is produced in the zona fasciculata under pituitary control. These conclusions were based largely on differences in structure and lipid content following experimental procedures. In some species, however, the zona glomerulosa is less well developed than in the rat and contains less visible lipid normally. In certain species, e.g., the hamster (Peczenik, '44; Alpert, '50), and the ruminants Brown et al., '37), there is scarcely any stainable lipid and very little cholesterol in any part of the adrenal cortex.

Alpert ('50) observed that hormone injections produced effects in the lipid-poor adrenal of the hamster that differed in certain respects from those reported by other investigators in the lipid-rich adrenals of the guinea pig and rat.

A functional interpretation of these species differences constitutes a challenging problem and one that must be solved before the fundamental nature of the secretory processes in the adrenal is adequately understood. There is evidence that species differences in adrenal function exist. Certain animals survive adrenalectomy much longer than others. In the opossum, the adrenal is apparently not so important in the body economy as in other mammals for the adrenalectomized opossum will survive for weeks or months with little or no sign of adrenal insufficiency (Hartman et al., '43); and since injections of the sodium hormone lead only to a temporary or moderate retention of sodium it is doubtful whether its adrenal produces a sodium hormone (Smith et al., '43). It has been demonstrated also (Hartman et al., '44) that the elasmobranch interrenal does not influence electrolyte metabolism and although it may not produce a sodium hormone it is probably necessary for normal carbohydrate metabolism. Such functional differences, however, have not been correlated with specific structural variations.

In view of these puzzling conditions, it is our judgment that a much broader basis for correlating adrenal structure and function is required. We have undertaken, therefore, a comprehensive comparative study of the histology, cytology

and histochemistry of the adrenal for the purpose of determining as precisely as possible the nature of the similarities and differences manifested by the adrenals in the various vertebrate groups. By this approach we hope to discover particularly favorable and critical types for experimental and physiological studies. Our initial efforts have been expended chiefly on the collection, preparation and study of the adrenals of birds, for with the exception of investigations on the fowl and pigeon, very little work has been reported on this large and diverse vertebrate group.

In a general survey of 420 species of birds we have found the adrenals of certain ones especially suitable for histological and cytological study. The adrenal of the Brown Pelican (*Pelecanus occidentalis*) is of particular interest as it presents several striking and distinctive features. It is heavier in proportion to body weight (Hartman, '46) and has a higher proportion of interrenal cells which are larger, more regular in form and arrangement, and exhibit a more marked structural and functional polarity than any other vertebrate adrenal thus far examined.

MATERIALS AND METHODS²

The adrenals of 40 adult males and 43 adult females were available for study. Several of the males possessed enlarged testes and a number of females were taken in the egg laying period. The adrenals, in most instances, were hastily removed in the field, bisected transversely and placed promptly in the fixing fluids, consequently they were not weighed or measured nor were the surface relations and the number and relations of the main adrenal vessels recorded.

Several fixing solutions were used but those routinely employed were 10% formalin and Helly's fluid. The time of fixation ranged from 6 to 18 hours. Some of the material fixed in the latter was treated from 4 to 8 hours with 2% osmium

² We are very much indebted to Mr. Richard Archbold of the Archbold Biological Station, Florida, for his personal assistance in the collection of material and for the use of the facilities of the Archbold Biological Station.

tetroxide after washing, and some was mordanted for several days in 3% potassium bichromate. The material fixed in Helly's fluid was embedded in paraffin and sectioned transversely at $5\ \mu$ for cytological study. Thicker sections were also prepared for topographical purposes. To obtain a three-dimensional concept of the organization pattern of the structural elements, one gland was cut serially in the transverse plane and blocks from several others were cut serially in a plane tangential to the surface. Thick ($30\text{--}50\ \mu$) unstained sections cut in vertical and tangential planes were examined for the same purpose.

Various staining methods were employed but the hematoxylin and eosin, Masson's trichrome, iron hematoxylin, and acid fuchsin light green stained preparations were the most useful. Frozen sections of the formol-fixed material were treated with Sudan black or Sudan orange to determine lipid content and distribution. The Schultz test for cholesterol was applied routinely. Reticular fibers were stained by the Wilder method and elastic and collagen fibers by Kornhauser's "Quad" stain. The staining methods employed are described by Cowdry ('48).

OBSERVATIONS

Topographical features. A membranous sheath of condensed areolar tissue in which the adrenal is embedded and connected to surrounding parts, is observed in sectioned material as a pericapsular layer of varying thickness and texture. Branches of the main adrenal vessels and nerves ramify in this layer over the periphery of the gland and penetrate the true capsule at various points. On one surface a thickening consisting of compactly arranged collagenous fibers interwoven with some smooth muscle fibers, encloses the extrinsic ducts of the testis or their homologues in the female (fig. 2). Elsewhere the pericapsular sheath is composed of a loose texture of fibers, predominantly collagenous with a few intermingled elastic and reticular fibers.

The capsule proper is composed of a dense felt-work of reticular fibers. Collagenous fibers are few and limited to the external surface of attachment to the overlying pericapsular layer. Elastic fibers are present in the walls of the larger capsular vessels. Reticular fibers from the internal capsular surface pass inwards and contribute to the formation of the stromal networks which envelop and support the parenchymal elements (fig. 6). Several septa involving both capsular and pericapsular tissue penetrate deeply into the glandular substance but their number and extent are insufficient to partition the gland into lobules.

The glandular parenchyma is supplied by two types of arteries and drained by two types of veins. The main adrenal arteries branch freely in the pericapsular sheath. A few large branches pass directly by way of the septa to the central region of the gland where their terminals connect with central venous sinuses (fig. 8). Many smaller arterial branches ramify in the pericapsular sheath over the glandular surface and enter the capsule at numerous points to form an arteriolar plexus. From this plexus vessels of minute size supported by networks of reticular fibers penetrate between the glandular units only a short distance before terminating in peripheral venous sinuses.

The central sinuses drain into several large venous channels which pass outward either in septa or directly through the parenchyma to the periphery where after receiving large capsular tributaries, they empty into larger veins in the pericapsular sheath (figs. 2 and 8). Peripheral sinuses connect directly with capsular veins at many points over the entire glandular surface (fig. 4). In comparison with the capsular arteries, the capsular veins are unusually large and appear as endothelial lined channels in the capsular connective tissue. The differences in vascular pattern between the peripheral and central regions of the gland, together with certain topographical and cytological differences to be described later, suggest regional variations in functional activity.

Several large ganglionated nerves are located in the pericapsular tissue. Their branches enter the capsule and form a very rich capsular plexus. Numerous ganglia some of considerable size are also present in the capsule. From the capsular plexus bundles of nerve fibers enter the parenchyma at various points over its surface. The larger bundles, frequently with chromaffin cells and occasionally with ganglion cells scattered along their course penetrate to the central areas. Some large bundles pass inward in the septa. The nerve fiber bundles are readily traced in serial sections to the chromaffin masses. The majority of the fibers are medullated as they are colored by the Schultz reagent.

Chromaffin cells singly and in small clumps are commonly found in the capsule. Groups of them are often seen within the capsular ganglia. They are more numerous at the interface between the capsule and the underlying glandular parenchyma.

Interrenal cells in small nodular and laminar formations are found also within the capsule. In several specimens they were observed within capsular ganglia (fig. 7), and in the connective tissue thickening enclosing the genital ducts. The presence of cortical (interrenal) cells in the capsule of the mammalian adrenal has been frequently reported. It has been concluded that these intracapsular cortical (interrenal) cells differentiate from capsular connective tissue cells and subsequently move inward and fuse with the glandular parenchyma. Elias ('48) has recently discussed the evidence for the concept that the capsule represents a region of appositional growth.

The glandular parenchyma consists of intermingled masses of interrenal and chromaffin cells, a pattern which distinguishes the avian from the mammalian adrenal in which the interrenal component forms the cortex and chromaffin elements the medulla.

It differs, however, from most avian adrenals in possessing relatively a much smaller amount of chromaffin tissue, the chromaffin masses being small, few and widely scattered

among the larger and more abundant interrenal masses (fig. 1). Consequently, it is the arrangement and form of the interrenal masses that characterize the gland structurally and account for its topographical similarity to the mammalian cortex.

Of the avian adrenals we have examined thus far, the Brown Pelican possesses the largest proportion of interrenal tissue. In the pigeon, according to the measurements of Miller and Riddle ('42), the interrenal component constitutes 65, the chromaffin tissue 32 and the stromal and vascular constituents 3% of the gland. We have made no exact measurements but from a comparison of sections of the two species it would appear that the interrenal component comprises from 85 to 90% of the glandular parenchyma in the adrenal of the pelican.

A remarkable topographical feature, which we have not observed as clearly in any other avian adrenal, is the organization of the interrenal component into three readily recognized zones which correspond in position and general appearance, but not in relative proportions, to the zona glomerulosa, zona fasciculata and zona reticularis of the mammalian adrenal (figs. 1 and 3). For the sake of convenience and the avoidance of new terms the mammalian terminology is adopted.

The zona reticularis is relatively much wider and the zona fasciculata much narrower than in the mammal, but the proportionate width of the zona glomerulosa is very similar to that found in the guinea pig. The average widths of the three zones, measured on transverse sections from the middle regions of 10 glands were: glomerulosa, 0.17 mm; fasciculata, 0.32 mm and reticularis 2.73 mm (the last measured from the inner border of the fasciculata of one side to that of the opposite side). Observed with low magnification (20 $\times$) the zona glomerulosa and the zona fasciculata, together constitute a narrow, denser peripheral portion of the gland and the zona reticularis a relatively larger central part of looser texture.

The zona reticularis appears in sections, regardless of the plane of cutting, to be composed chiefly of convoluted strands of interrenal cells which branch and anastomose freely to form a network, in the meshes of which lie the vascular channels and the widely scattered clusters of chromaffin cells (figs. 3 and 10). Each strand appears as a double row of columnar cells with nuclei located in a double line in the axis of the strand (figs. 1 and 13). At widely scattered places oval or elliptical groupings, resembling those in the zona glomerulosa are encountered. Shrinkage with collapse of the poorly supported blood sinuses during fixation and preparation of sections is more pronounced than in the other zones.

The zona fasciculata in transverse sections vertical to the glandular surface presents a radial pattern of parallel cell columns as in mammals (figs. 1 and 3). It varies in width in different regions of the same gland and is more pronounced in some glands than in others. Although a sexual variation was not clearly established it appears to be better developed in the larger glands, which in our collection were more frequently taken from sexually active males and ovulating females. It is usually wider on the side next to the genital ducts. Occasionally it is extremely narrow or even absent in which case the glomerulosa appears wider (fig. 4). Anastomoses and branchings of the columns are infrequent peripherally but more numerous centrally where it passes without a sharp line of demarcation into the zona reticularis. The columns like the strands of the reticularis appear in sections as double rows of cells with the nuclei axially placed.

The zona glomerulosa is usually well defined. It is composed of circular, ovoid or arched shaped masses of variable size; the larger ones appear to be the peripherally expanded ends or the bent and reflected portions of the fasciculate columns, the smaller ones, knob-like projections of the larger ones. These formations when sectioned transversely show a radial cellular arrangement resembling the tubules of an exocrine gland (figs. 9 and 12).

Cytological features. In the literature of the avian adrenal the interrenal units have been described as cords, strands or columns. After critical examination of serially sectioned glands in various planes, it is concluded that the basic three dimensional form in the pelican adrenal is a cellular cord. Tangential sections which cut the cords in the glomerulosa and fasciculata zones transversely show the cell organization clearly (fig. 9). An interrenal cord consists of a single row, usually of from 20 to 50 tall wedge-shaped cells arranged in a radial pattern similar to an exocrine gland tubule prior to canalization.

The tapered ends of the cells form a solid central core and the broadened distal ends a mosaic pattern in the peripheral surface facing the sinuses (fig. 14). The nuclei are regularly situated near the central poles of the cells and the bulk of the cytoplasmic masses containing virtually all the organoids and inclusions lies between the centrally placed nuclei and the peripheral surface. The general form of the cells is that of a 5 or 6 sided prism. The manifest polarity and regular arrangement of the cells in the cords shown diagrammatically in figure 14, are impressive cytological features.

Occasionally central cavities resembling the lumina of gland tubules are observed especially in the zona glomerulosa (figs. 5 and 9). These central cavities have been examined critically since they have been described in the mammalian adrenal. Lucadou's ('38) concept of a functional gland unit, which he terms the epi-nephron is based on the observation of such gland-like cavities. In our material, they are, in our opinion, simply artifacts. The central ends of the cells are probably subject to more rapid degeneration, and hence more difficult to fix properly. They may result also from the shrinkage of dehydration since they are less frequently observed in frozen sections.

The strand-like arrangement of the interrenal cells observed in thin sections, in the zona reticularis especially, results from the lengthwise cutting of cords. In the zona reticularis

where the cords are not regularly oriented in reference to any plane, but loop, branch and anastomose in an intricate manner, longitudinally cut cords are the rule regardless of the plane of sectioning of the gland. However, oval and elliptical units showing the typical radial pattern of cords cut transversely can always be found in the zona reticularis (fig. 10) and such groupings are seen predominantly in the zona glomerulosa and zona fasciculata in sections cut tangential to the surface of the gland.

It is difficult to determine accurately the proportion of the cells of a cord contacting a blood sinus. For the distal surface of every cell to reach a sinus wall a radial orientation of cells in reference to the sinus would be required. This arrangement is not the general rule, although, in the reticularis zone there is a tendency toward such a relationship. The cords in this zone convolute, and coil to such an extent that often a sinus appears to be enclosed by a wall of radially arranged cells (fig. 10).

Lipid droplets are the most conspicuous inclusions of the interrenal cells. Although the number varies, no cells are completely devoid of them. They are always found between the nucleus and the free border. In most cells in the glomerulosa and outer fasciculata zones, the cell body between the nucleus and the free border is packed with droplets of uniform size. Such cells, in sections prepared by the paraffin method, present the classic appearance of spongiocytes (figs. 12 and 15). In the zona reticularis they are less heavily loaded. In cells with relatively little lipid the droplets are often grouped in a goblet-like expansion. The distal or peripheral borders of such cells are almost completely devoid of droplets (fig. 13). There are of course transitional forms between the two extremes. The lipid droplets are sudanophilic, osmiophilic and Schultz positive, the last indicating the presence of cholesterol. The heavier lipid content of the cells in the periphery of the gland is illustrated in figure 11.

Mitochondria were best revealed in the material mordanted in bichromate and stained with acid fuchsin and fast green.

Their number and distribution vary inversely with the lipid content (compare figs. 15 and 16). In the cells with fewer lipid droplets (fig. 16), they are heavily distributed throughout the cytoplasm; being most abundant in the extreme distal ends where they consist of closely packed granules and short rods. Nearer the nucleus in the area of lipid concentration, they are fewer in number, granular in form and occupy the cytoplasmic interstices between the lipid vacuoles. In the lipid-laden spongiocyte, the mitochondria are markedly reduced in number and confined mostly to the narrow spaces between the lipid droplets (fig. 15). In Masson stained preparations both the mitochondria and the hyaloplasm of the cells poor in lipid are colored with the fuchsin, consequently such cells are markedly fuchsinophilic. The cells loaded with lipid are only faintly stained with the fuchsin and appear light and clear.

Very deeply staining fuchsinophil cells of a different nature are also encountered. They are more abundant in some glands than others and occur most frequently in the zona reticularis, although they have been seen in the fasciculata and the glomerulosa. They are markedly siderophilic in the iron hematoxylin stained preparations and also basophilic when stained with toluidin blue. Since this basophilic material is removed by ribonuclease, it is probably nucleoprotein. Such cells usually possess a few lipid droplets, and masses of clumped mitochondria. Their nuclei are pycnotic. Similar cells have been reported in mammalian adrenals and identified as expended, degenerating cells.

In none of our preparations were we able to identify positively the Golgi apparatus. It was not distinguishable in our osmicated material which we had hoped to use for its study. Attempts to impregnate it in other material were unsuccessful.

The fuchsin spheres observed by Miller and Riddle ('42) in the pigeon were noted occasionally, but neither spherical non-lipid vacuoles nor pigment granules, also described in the pigeon by the same authors, were observed in the interrenal cells of the pelican.

The chromaffin cells present no peculiar cytological features. They are clumped together in small groups or attenuated strands without orderly arrangement. They are relatively large, irregularly spherical, or angular shaped and stain darker than the interrenal cells with most stains. Some give stronger chromaffin reactions than others. In general, the cells in the peripheral masses give a stronger chromaffin reaction than those situated centrally, which is the reverse of the condition reported by Müller ('29) in the pigeon and fowl. In addition to the chromaffin granules, the cytoplasm contains numerous fine mitochondria, but few lipid inclusions. Nerve fibers can be traced to most of the cell groups, as they are very plainly revealed by the Schultz reagent.

DISCUSSION

It is unfortunate that the Brown Pelican is not available as a laboratory subject. In comparison with the fowl and pigeon, commonly used in laboratory studies, the interrenal component of its adrenal exhibits topographical and cytological features that are clearly defined and hence easier to evaluate in terms of function. From this standpoint, it would be a very desirable form to use in experimental studies designed to determine the fundamental nature of the secretory processes in the adrenal cortex.

The presence of a well defined zonation which clearly resembles that found in the mammalian adrenal is of unusual interest. Miller and Riddle ('42) suggest that the absence of a well defined zonation in the pigeon adrenal may be accounted for by the intermingling of interrenal and chromaffin elements. The fact that chromaffin tissue in the pelican is relatively much less than in most other birds supports this view. However a zonation has not been reported, to our knowledge, in the elasmobranch adrenal which consists solely of interrenal tissue. It seems more likely that zonation depends on specific patterns of growth, differentiation and function.

An indication of zonation has been reported in the fowl and pigeon. Latimer and Landwer ('26) found in the fowl

a peripheral grouping of enlarged cortical masses which reminded them of the zona glomerulosa of mammals and Müller ('29) noted peripheral cortical masses, clearly distinguishable from the central masses by differences in size and arrangement. In the pigeon, the latter observed radially disposed cortical columns. Miller and Riddle ('42) remarked that gross differences between areas of cortex which they attributed to a maturation gradient, occur in the pigeon.

The latter authors believe that the current escalator concept of the life history of mammalian cortical cells (Zwemer et al, '38), is valid also for the pigeon. We could find no positive evidence for this concept in the pelican adrenal. Senescent degenerating cells with pycnotic nuclei and very deeply staining cytoplasm, were found in varying numbers and although usually more abundant in the reticularis, were observed in the fasciculata and occasionally in the glomerulosa. Moreover, no cells in mitosis or amitosis were identified even in glands with large numbers of degenerating cells. We could find no conclusive evidence for a transformation of capsular cells into cortical cells. The boundary between the capsule and the peripheral interrenal masses is usually sharply defined and no convincing evidence of an incorporation of transitional cells into peripheral interrenal units was found. Small clusters of cells frequently seen within and just beneath the capsule were positively identified as chromaffin cells.

In many glands groups of interrenal cells in the form of nodules and lamina were found in the capsule, even within capsular ganglia and among the genital ducts. Often such groups when traced in serial sections were found to be connected with subcapsular interrenal units. Elias ('48), Gruenwald ('46) and others have reported similar conditions in mammalian adrenals and have concluded that such masses arise in the capsule from indifferent cells and move into the cortex. We could find no positive evidence that such capsular masses in the pelican arise in this manner. In all instances the cells of these masses possessed lipid droplets and

appeared fully differentiated. The connections of the peripheral interrenal units with the capsular interrenal masses might be considered as evidence of outgrowth from subcapsular units with as much justification as to consider them as evidence of incorporation of units arising independently in the capsule, in fact more so in the case of the pelican adrenal, since the capsular interrenal cells present the same cytological characteristics as the subcapsular interrenal cells. Indeed deeply staining senescent cells were sometimes found in the capsular interrenal masses. The problem of prenatal as well as postnatal growth and cell replacement merits further study in birds where the adrenal capsule is thin and mitoses have been infrequently observed.

Another feature of special interest and functional significance in the pelican adrenal is the organization of interrenal cells into solid cord-like or cylindrical formations, composed of a single layer of elongated radially arranged cells whose distal ends form the surface facing the blood channels and opposite (proximal or central) ends converge to form the axial cores. This regular disposition of the cells in the cords establishes a very distinctive and significant type of structural and functional polarity not exhibited in the adrenal gland of any other species, bird or mammal, with which we are familiar.

Since the pelican belongs to a group of birds that is placed near the base of the avian branch of the evolutionary tree, it is tempting to consider this simple organizational pattern as a primitive or generalized feature and to conclude that conditions in higher birds are modifications of this pattern. Our present information regarding the comparative histology of the avian adrenal is too fragmentary to advance this as a general principle. In fact a good case can be made for the contrary view that the pattern exhibited in a particular species represents functional adaptations without evolutionary significance.

According to Müller ('29) the interrenal cells of the adult fowl are arranged in strands of two to three layers of col-

ummar cells which frequently show an ill-defined radial arrangement. In the chick, however, spherical and oval cells comprise the strands. Kar ('47) described the interrenal masses in fowls, 22 months of age, as being composed of round, oval, ovoid, hexagonal and even octagonal cells. In the pigeon Müller ('29) described strands formed by two or more rows of cuboidal or columnar cells, with nuclei regularly placed in the ends of the cells facing the blood channels. Miller and Riddle ('42) found in the pigeon a tendency for the interrenal strands to be composed of a double row of cells with their long axes in the transverse plane of the strand. In the more peripheral strands, nuclei of cells, which they believed to be younger, were located in the end directed away from the edge of the strand and the blood supply, but in the central region of the gland, the nuclei lay at the end directed toward the blood supply. They are of the opinion that the young cells at the periphery move toward the center as they mature, and claim that nuclei during the aging process shift to opposite ends of the cells. The functional significance of these variable types of polarity presents an interesting and fundamental problem in the histophysiology of interrenal cells.

In the type of cell polarity found in the pelican adrenal it is obvious that all direct exchange between the cortical cells and the blood stream must occur at their distal surfaces only, since their lateral and constricted basal surfaces are in relation to contiguous interrenal cells. It is apparent also that the characteristic presecretory processes occur in the body of the cell between the nucleus and the free surface for in this region are found most of the mitochondria, and the lipid droplets accumulate there. The nucleus is placed very close to the base of the cell which it almost fills; more centrally the cytoplasm is drawn to a point or wedge. This type of polarity is uniformly found in all interrenal configurations. However an interesting condition occurs in the reticularis zone where the cords branch, anastomose and coil. Here the distal ends of the cells are frequently radially oriented in reference to

the sinuses. This attempt at a double orientation, so to speak in reference to both cord and sinus may account for the more complex and irregular pattern of the cortical cords in this region. It suggests also a greater functional efficiency, which is indicated also by less lipid and more abundant mitochondria. However, the roles of lipid, mitochondria and the Golgi apparatus in cortical cell function are still poorly understood. Miller and Riddle ('42) concluded from the results of their experimental studies on the pigeon that cell lipid decreased and mitochondria increased in interrenal cell hyperactivity while the reverse occurred in hypoactivity. Applying these findings to the normal pelican adrenal, it would appear that the cells of the reticularis zone are more active than those of the outer zones, since the latter are more intensely colored with the fat stains. Although the fat droplets and mitochondria were both clearly demonstrated in our osmicated preparations stained with acid fuchsin, and light green, we were unable to determine whether or not the mitochondrial organoids transform into lipid droplets. We are inclined to think that they do not, but the finding of Miller and Riddle ('42) that there is an inverse relation between lipid and mitochondrial content in the pigeon is certainly true also for the interrenal cells of the pelican adrenal. We favor the view that the mitochondria are transient elements, concerned perhaps with providing enzymes for synthetic processes. During the lipid storage phase they are reduced in number but appear again in larger numbers when cellular activity is increased.

SUMMARY AND CONCLUSIONS

1. In a general microscopic survey of the adrenals of many species of birds, it was discovered that the adrenal of the Brown Pelican, *Pelecanus occidentalis*, although consisting of intermingled masses of interrenal and chromaffin cells as in other birds, resembles the mammalian adrenal in certain respects and presents other features peculiar to itself.
2. It has less chromaffin tissue proportionately than any other avian adrenal thus far examined.

3. The predominant interrenal masses are distributed in zones which correspond to those of the mammalian adrenal. However, the zona reticularis forming the entire central mass of the gland is relatively much broader and the fasciculata relatively much narrower than in the mammalian adrenal.

4. The interrenal masses are organized into cords consisting of a single row of tall columnar cells radially arranged like those of a gland tubule, with their broader ends forming the surface facing the blood channels and their opposite, narrower, nucleated ends converging to form the solid central cores of the cords.

5. The uniformity of this cellular pattern bestows upon the cortical cells a polarity not exhibited in the adrenal gland of any other species, bird or mammal, with which we are familiar.

6. Lipid droplets which are the most characteristic inclusions of the interrenal cells and perhaps represent what corresponds to secretion granules in the acinar cells of exocrine glands, are located between the nucleus and the surface facing the blood stream.

7. The interrenal cells in the zona glomerulosa and fasciculata contain more lipid than those in the reticularis.

8. There is an inverse relationship between the lipid and mitochondrial content of interrenal cells.

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PLATES

PLATE 1

EXPLANATION OF FIGURE

1 Drawing of a portion of a transverse section of the adrenal gland of the Brown Pelican. showing topographical features, Cap., capsule; Z.G., zona glomerulosa; Z. F., zona fasciculata; Z. R. zona reticularis (only outer portion of the very broad zone included); C.M. chromaffin masses.

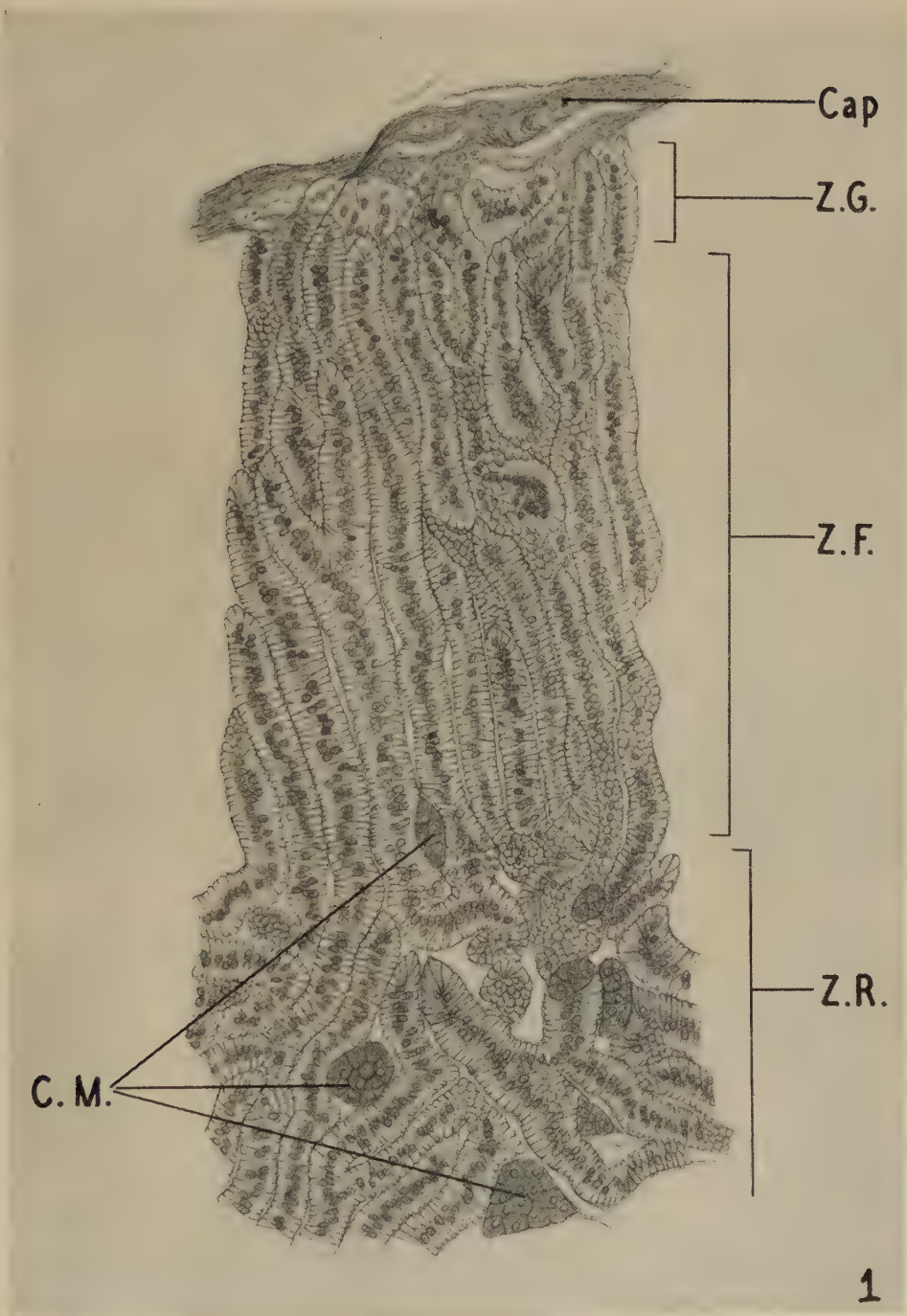


PLATE 2

EXPLANATION OF FIGURES

All figures are unretouched photomicrographs of sections of the adrenal gland of the Brown Pelican.

2 Transverse section showing general topographical features, Masson stain. $\times 16$. E.D., efferent genital ductules, male; P.T., pericapsular connective tissue; A, artery; V, vein; V.C., venous channels.

3 Field from a transverse section showing zonation. Masson stain. $\times 72$. Cap., capsule; Z.G., zona glomerulosa; Z.F., zona fasciculata; Z.R. zona reticularis

4 Field from a transverse section showing a peripheral sinus, P.S., opening into a large capsular vein, C.V. Masson stain. $\times 63$. Note that the zona glomerulosa is well defined but the zona fasciculata is hardly recognizable. Compare with figure 3.

5 Field from a transverse section showing central gland-like cavities, G.C., in the interrenal masses. Masson stain. $\times 112$.

6 Field from a transverse section showing the reticular fibers of the capsule, Cap., and the networks of reticular fibers, S, enclosing and supporting the interrenal units and peripheral sinuses, P.S. Wilder's reticular stain. $\times 345$.

7 Field from a transverse section showing masses of interrenal cells within a capsular ganglion; I.R., interrenal cells; N.C., nerve cells, Cap., capsule. Compare the state of differentiation of these masses with those of the zona glomerulosa, Z.G. Masson stain. $\times 216$.

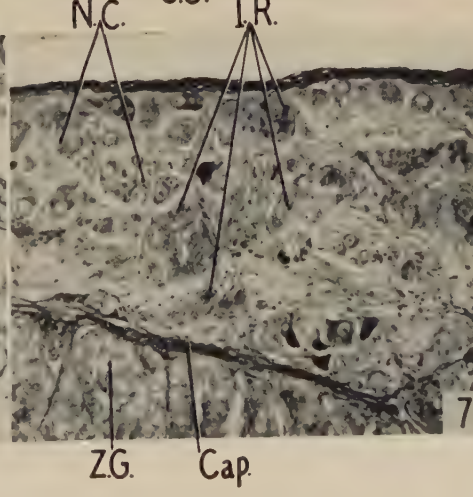
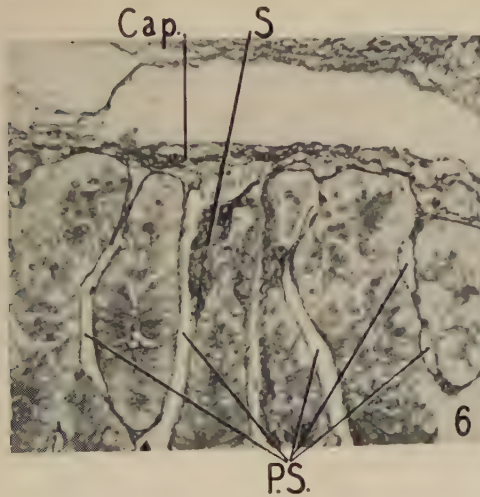
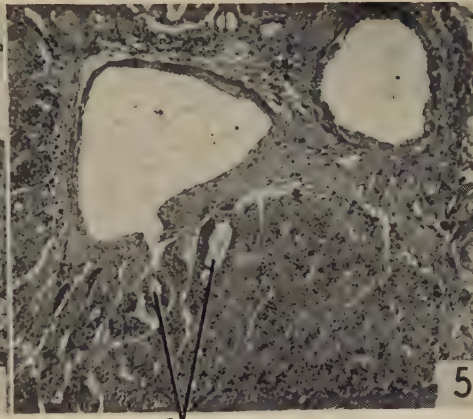
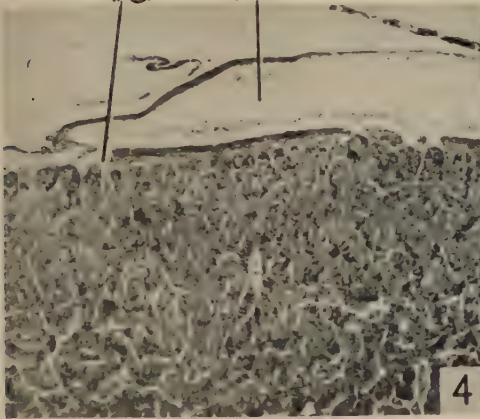
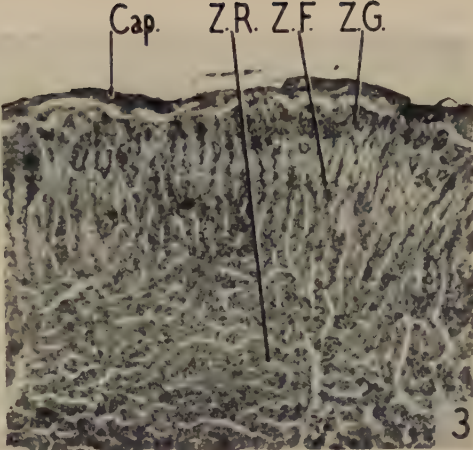
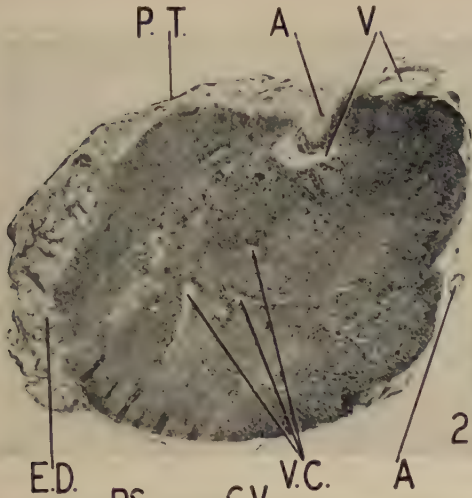


PLATE 3

EXPLANATION OF FIGURES

All figures are unretouched photomicrographs of sections of the adrenal gland of the Brown Pelican.

8 Field selected to show a cross section of a septum which has penetrated deeply into the central region of the gland. A, artery; V, vein. Masson stain. $\times 120$.

9 Field from a tangential section showing the interrenal masses of the zona glomerulosa, Z.G., and the zona fasciculata, Z.F., sectioned transversely and revealing the cord or cylindrical form of the interrenal masses. Masson stain. $\times 93$.

10 Field of the zona reticularis, selected from a thick (20μ) section stained with Sudan black. $\times 88$. Nuclei, sinusoidal spaces and chromaffin masses are unstained, lipid droplets of the interrenal cells deeply colored. At L, the cords are cut longitudinally and appear on section as strands of two rows of cells with the unstained nuclei located in the axes of the strands; at T, the cords are sectioned transversely and appear as oval or circular formations with the unstained nuclei located centrally. At C, an interrenal cord coiled around a venous sinus, V.S.; appears in section as a ring-like formation. Note the radial arrangement of the inner layer of cells.

11 A field selected from a thick (20μ) frozen section stained with Sudan orange. $\times 85$. Note the high lipid content of the zona glomerulosa, Z.G.

12 A transverse section of a cord of the zona glomerulosa, showing lipid rich, mitochondria poor, spongiocyte, type of interrenal cells. The fine mitochondria are few and located in the interstices formed by the lipid droplets. Zenker-formol fixation. Acid fuchsin, light green stain. $\times 1067$.

13 A field selected from a longitudinally sectioned cord of the zona reticularis, showing the mitochondria rich, lipid poor type of interrenal cells. Compare with figure 11. Zenker-formol fixation. Acid fuchsin, light green stain. $\times 1067$.

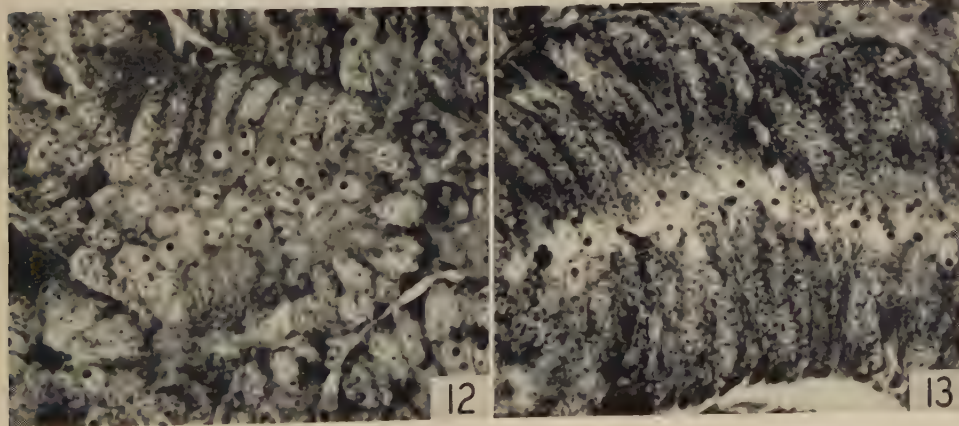
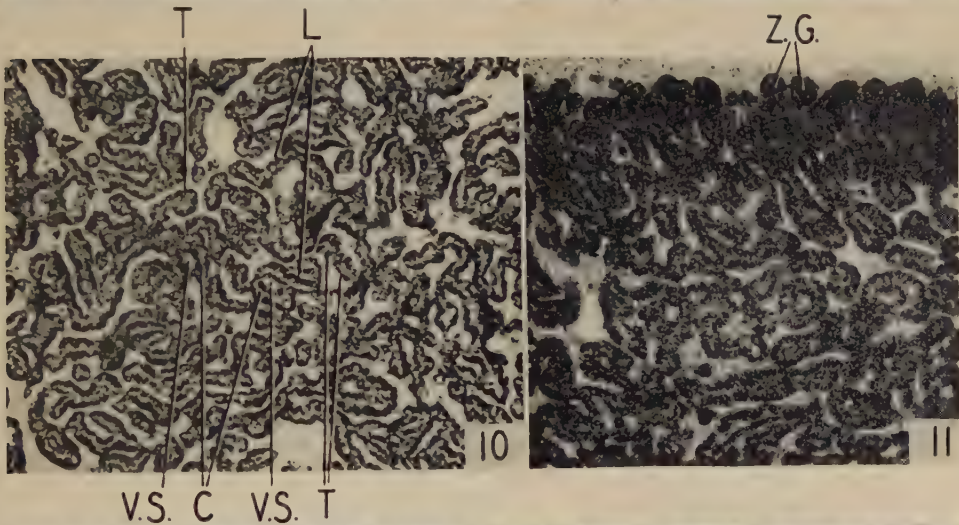
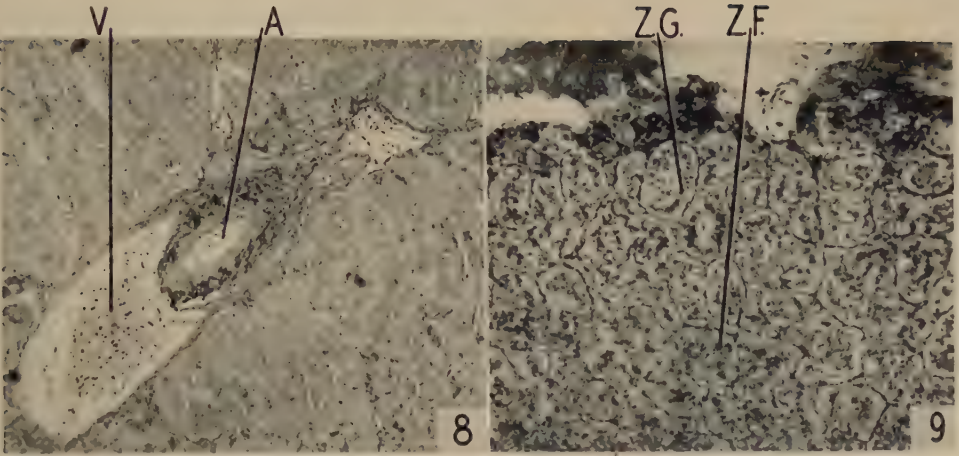


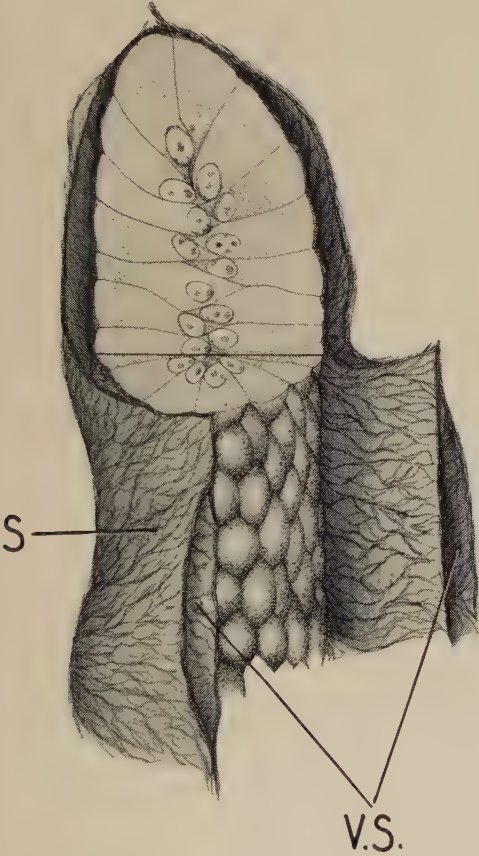
PLATE 4

EXPLANATION OF FIGURES

14 Graphic three-dimensional illustration of the arrangement of the cells of an interrenal cord. S, encircling stromal sheath of reticular tissue; V.S., venous sinuses.

15 A lipid rich, spongiocyte type of interrenal cell found chiefly in the glomerulosa and outer fasciculata zones. Acid fuchsin, light green stain. The mitochondria are reduced in number and confined to the narrow cytoplasmic strands between the lipid vacuoles.

16 A mitochondria rich, relatively lipid poor interrenal cell from the zona reticularis. Acid fuchsin, light green stain.



14



15



16

ALKALINE GLYCEROPHOSPHATASE IN THE DEVELOPING ENDOCRINE PANCREAS OF THE ALBINO RAT¹

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ELEVEN FIGURES

INTRODUCTION

An increased knowledge of fundamental biological processes has pointed out the necessity of re-examining our basic concepts in biochemical terms. No longer can the differentiative processes be considered in terms of morphological change alone. They must be viewed more specifically in terms of the qualitative and quantitative alterations in the chemistry of the cell or tissue which occur as the differentiative mechanism asserts its effects. This study concerns itself with the alterations of alkaline phosphatase which occur during the development of the endocrine portion of the pancreas of the albino rat.

MATERIALS AND METHODS

The entire developmental history of the cells of the endocrine portion of the pancreas has been studied. Embryos were collected at half day intervals between the 12th and 14th days of gestation, and at daily intervals between the 14th day and full term. Postnatal animals were sacrificed at one

¹ This work has been supported by a cancer research grant from the Indiana Elks Association. The writer wishes to express his gratitude to Dr. Theodore W. Torrey, chairman of the Department of Zoology, for his helpful suggestions and encouragement in this study, to Dr. Edwin N. Kime, professor of Clinical Anatomy, and Dr. Richard L. Webb, chairman of the Department of Anatomy, who have kindly provided the facilities necessary for the work, and to Miss Janette Bleicher for her constant assistance in the laboratory.

and two days of age and at 5 day intervals between the 5th and the 20th days. At least two animals were studied for each age group. Six to 8 animals were used for the developmental stages in which significant changes in the enzyme content of the cells occurred. No significant differences were observed between the two sexes.

The histochemical technique devised simultaneously by Gomori ('39a) and Takamatsu ('39) was used with some modification to detect the presence of the enzyme in the tissues. The younger embryonic and fetal specimens were fixed, imbedded, and sectioned in toto. Older fetuses (19 through 22 days) were eviscerated, and the mass of organs was fixed, after which the pancreases were dissected out, imbedded, and sectioned. The pancreases of postnatal animals were dissected out prior to fixation. Cold 80% alcohol was used as a fixative for all specimens. The duration of fixation varied from 6 to 12 hours. The tissues were dehydrated, cleared, and imbedded in paraffin in the usual manner. Serial sections were cut, varying in thickness from 6 μ up to 10 μ . Decalcification of the whole embryos was not necessary. The sections were incubated for two to three hours at a temperature of 37°C. in a substrate of the following composition:

2% Sodium glycerophosphate	25.0 ml
2% Sodium barbital	25.0 ml
2% Sodium chloride	5.0 ml
2% Magnesium sulfate	2.0 ml
Distilled water	50.0 ml

Control sections were incubated in a substrate from which the glycerophosphate had been omitted. Following incubation the tissues were rinsed in a .5-1% solution of calcium chloride, placed in a 2% solution of cobalt chloride for 5 minutes, rinsed thoroughly in distilled water, placed in dilute yellow ammonium sulfide (1 ml per 60 ml distilled water) for three minutes, rinsed in tap water, dehydrated, cleared, and covered. The sections were either counterstained with erythrosin or with azocarmine-fast green or were not counterstained at all.

INTERPRETATION OF THE SECTIONS

A critical analysis of the technique will not be undertaken here since such analyses have been made by Gomori ('41), Danielli ('45-'46), Emmel ('46), and Stafford and Atkinson ('48). The total of histo- and biochemical evidence indicates that the technique is a reliable index of the location of the enzyme in various tissues. The recent studies of Rabinovitch and co-workers ('49) and of Palade ('50) on the acid phosphatase, however, would indicate that such a technique as this may not be relied upon for accurate intracellular localization of the enzyme.

It must be emphasized that at best the technique is only qualitative, and in no way gives any accurate information concerning the absolute quantity of phosphatase within the cells and tissues. According to Stafford and Atkinson only 25-30% of the *in vivo* phosphatase, measured by biochemical techniques, remains after the tissues have been subjected to this treatment. In addition, certain subtle variations in technique may further reduce this to a smaller percentage, but when used consistently, the technique does give an indication of the relative amount of the enzyme in the cells and tissues as it varies according to the developmental stage of the embryo. The deposits of cobalt sulfide have been taken as indicative of the location of the enzyme, and quantitative changes are interpreted as an increase or a decrease with respect to other stages of development. It must be stated that the problem of selective removal of intracellular phosphatase and other substances possibly necessary for phosphatase activity by the dehydrating and clearing agents and the selective affinity of certain tissues for the precipitated calcium salts might possibly cloud the true picture.

Minimal phosphatase activity has been designated as such, and is characterized by only a small amount of sulfide precipitate in the nuclear membrane and nucleolus with none in the cytoplasm. Cells which exhibit a moderate precipitate in the nuclei with none in the cytoplasm have been indicated as having "1 +" activity. Maximal activity has been desig-

nated as "5 +," and is characterized by a dense precipitate in both cytoplasm and nucleus to the extent that individual cell outlines are difficult to determine. Gradations in cellular activity between "1 +" and "5 +" have been assigned values from "2 +" to "4 +."

OBSERVATIONS

Part I. Prenatal development

At 12 days. The dorsal pancreatic anlage consists of a small bud projecting from the foregut. The cells in the rostro-dorsal portion of the bud exhibit a 3 + activity (fig. 1), whereas those in the caudal portion and those in the base near its attachment to the foregut exhibit only minimal activity. There is no sharp line of demarcation, either morphologically or with regard to phosphatase activity, between these two cell types.

At 12½ days. The dorsal pancreatic lobe consists of a diverticulum into which the lumen of the foregut extends. Small groups of cells in the surface of the bud exhibit 3 + to 5 + activity. The remainder of the cells in the anlage exhibits only minimal activity. The number of cells per each reactive cell group varies from approximately 30 up to several hundred. Each clump of cells is characteristically located in the surface of the diverticulum. The largest of the cell groups tends to cause a slight bulge on the surface of the anlage. Of the cells in each group, those adjacent to the mesenchyme exhibit the maximal 5 + activity, whereas those along the deep surface of the cell group adjacent to the unreactive cells in the wall of the diverticulum exhibit an intermediate activity. The smallest of the reactive cell groups presumably are the more recently formed and accordingly are not set off from the general epithelium of the diverticulum as sharply in terms of phosphatase activity and morphological details as are the cells in the larger reactive cell groups. It is interesting to note that there is a degree of consistency in the location of the reactive cell groups in different embryos of the same age. This is

especially true of the largest of the reactive cell groups which is consistently located on the rostral surface of the bud near its base. This cell group forms a prominent bulge on the surface of the bud.

The ventral pancreatic anlage is present as a small bud of cells attached to the liver diverticulum. It exhibits only minimal activity throughout.

It will become increasingly evident in the description of subsequent stages that it is the phosphatase rich cells which develop into the endocrine portion of the pancreas, whereas it is the unreactive portion which branches and expands to form the exocrine pancreas. Hereafter, for convenience, the reactive cell groups will be referred to as the endocrine anlage and the unreactive portion as the exocrine anlage.

At 13 days. At this age the dorsal pancreas has increased in size, the walls of the diverticulum have thickened to obliterate the former lumen, and the cells of the mass, with the exception of the endocrine cells are becoming arranged to form anastomosing cords of cells (fig. 2). The entire mass of cells has assumed a somewhat lobulated appearance with the minimally reactive exocrine pancreas bulging slightly into the adjacent mesenchyme between the groups of reactive cells. These changes are an expression of the rapid growth and rearrangement of cells in the exocrine pancreas which precede actual tubulation of the cords. The ventral pancreas is a small but well defined cord of cells attached to the main stem of the liver diverticulum. It exhibits only minimal activity.

In the dorsal pancreas the large reactive cell groups have become more clearly delimited from the underlying exocrine pancreatic cells (fig. 2). The larger masses of endocrine cells have become more compact, so that a shrinkage space often separates these cells from the subjacent exocrine cells. A study of serial sections reveals that there may be three or 4, sometimes as many as 5 of these reactive cell groups in the surface of the lobulated primitive cord. They vary in size and shape; they may be individual spherical masses of

cells set in the surface of the cord; they may form a sheet of several cells in thickness covering perhaps one-fourth to one-third of the surface of the bud; or they may be arranged as a network of branching cords of cells projecting over a considerable portion of the primitive lobule.

At 14 days. During the 14th day there is a centrifugation of the exocrine cells so that the reactive endocrine cell masses tend to become buried in the central portion of the gland (fig. 3). In addition to the peripheral shift of the exocrine cords, there is an invasion of the primitive cord by the adjacent mesenchyme which tends to accentuate the lobulation. Some of the cords of cells are undergoing distinct tubulation. All of the cords of exocrine tissue are traceable to the center of the gland which represents the position of the original pancreatic cord.

The ventral pancreas has begun lobulation and is becoming more closely approximated to the dorsal pancreas. The dorsal pancreas at this age is still the larger of the two components.

The masses of reactive endocrine cells exhibit the same 5 + activity which has been noted in the 13 day embryo. They are located in the central portion of the gland and tend to be applied to the surface of the cords of exocrine tissue, and in some instances, to form a solid mass of reactive cells extending between cords of exocrine cells (fig. 3). In addition to the larger groups of cells in the central portion of the gland there may be a few small reactive cell groups located distinctly more peripherally in the exocrine cords. All of these reactive cell groups are very closely associated with the exocrine cords of cells and at no place have they lost contact with the parent exocrine anlage. The larger masses of reactive cells bulge into the mesenchyme which has penetrated between lobules. Rearrangement of the cells of the anlage and ingrowth of connective tissue are by far the most important changes which occur during the 14th day.

In the ventral pancreas there are a few small reactive cell groups. These groups of endocrine cells are not nearly as

prominent and numerous as are those in the dorsal pancreas, either at this stage or at any time later.

At 15 days. The dorsal and ventral anlagen are very intimately associated though complete fusion has not yet occurred. Both dorsal and ventral parts of the pancreas have undergone distinct tubulation so that relatively few untubulated cords remain. This tubulation conspicuously does not involve the reactive endocrine cell groups, though these reactive cell masses retain their continuity with the walls of the newly formed tubules. Most of the reactive cell groups remain centrally located where they are attached to the central tubules. In certain instances a group of endocrine cells may be seen actually to form a portion of the wall of one of the primitive tubules.

The number of individual endocrine cell masses at this stage of development is distinctly greater than at 14 days. This either represents a breakup and scattering of the primitive endocrine cell masses as the exocrine tubules draw apart, or else a progressive endocrine cell differentiation from new foci in the walls of the primitive tubules. The latter possibility is most likely. Such differentiation of endocrine cells from the walls of the primitive tubules will be described in detail as seen in the 16 day fetus.

At 16 days. Differentiation of endocrine cells from the primitive tubules is very evident by the end of the 16th day. The transformation of minimally reactive tubule cells into embryonic endocrine cells is represented by gradual changes both in morphological appearance and phosphatase activity. The initial changes which occur in the tubule cell as it undergoes differentiation into an endocrine cell are an increase in phosphatase activity of the nucleolus, an increased activity and number of distinct intranuclear fragments, and the appearance of a general haziness of the nuclear sap. These features characterize 1+ activity. As the cells further differentiate, the intensity of the nuclear reaction increases and the cytoplasm begins to show a moderate stainability (2+). The intensity of the reaction increases through the 3+ and

the 4 + stages until it reaches a maximum 5 + activity. Accompanying these changes in phosphatase concentration and localization there is a centralization of the nucleus and an enlargement of the cell.

The largest of the masses of differentiating endocrine cells are located in the central portion of the gland (fig. 4). More peripherally there are smaller reactive cell groups. The tubules from which these reactive cell groups have been derived exhibit 1 + activity. It is not uncommon to find a single cell within the wall of a tubule which exhibits 3 + to 5 + activity. In the central portion of the gland fusion of adjacent masses of 5 + cells derived from different tubules has occurred.

The foci of endocrine cell differentiation in the ventral pancreas present features similar to those seen in the dorsal pancreas, except that they are considerably fewer in number and less advanced.

At 18 days. By the end of the 18th day the masses of 5 + endocrine cells have become considerably more compact, and in some instances marked fusion of adjacent reactive cell groups has occurred. Thus, a larger islet cell complex consists of a bridge of intensely reactive cells extending between several tubules (fig. 5). Tubuloinsular cell transformation is still progressing as evidenced by the increased number of 2 + to 4 + reactive foci along the tubule walls and by the increased size and number of islet cell complexes.

In a few of the islet complexes a new cell type is present. These cells exhibit a considerable decrease in phosphatase activity, especially in their cytoplasm, and a concomitant increase in cell and nuclear size. The nuclei are round and centrally located. The appearance of this new cell type follows the extension of capillaries into the compact mass of phosphatase rich cells. Each of the cells exhibiting these changes abuts against a capillary on at least one surface.

At 19 days. By the end of the 19th day vascularization of the larger islet complexes is markedly advanced. The cells located in the central portion of the islet complex now ex-

hibit the characteristics of the new cell type described previously (p. 10). The intensely reactive bridge of compact cells seen at 18 days now consists of cords of large, centrally located cells separated by a capillary network (see fig. 6). The cells adjacent to the capillaries exhibit a marked decrease in phosphatase activity. The peripheral portions of the islet complex which are attached to the tubules are as yet uninvaded by capillaries and remain compact and intensely reactive (5+).

The morphology, phosphatase characteristics, and location of the central cells in the islet complex, when compared to the same features in the adult islet, identify the new cell type as the beta cell.

At 20 days. During the 20th day there is a continued rapid addition of cells to the existing islets as well as the formation of new islets. The larger islets more nearly exhibit the shape of the adult islets (fig. 6). Contact with the tubules, however, is maintained by way of compact, intensely reactive cell masses connecting the periphery of the islet to the tubule wall. That the beta cells are being differentiated from the tubules via the compact masses of intensely reactive cells rather than directly from the less reactive tubule cells is indicated in figure 6 where a well defined layer of connective tissue separates the mass of beta cells from the tubule wall.

At 21 days. During the 21st day the chief activity is apparently the differentiation of beta cells from the peripheral reactive cell masses attached to the tubules, as evidenced by the increase in size of the central masses of beta cells and a corresponding decrease in mass of the peripheral collar of reactive cells. The beta cells exhibit further clearing of the cytoplasm and continued loss of the enzyme activity in the nucleus. The nuclear membranes and nucleolus stain less sharply than previously and the entire nucleus exhibits a more homogeneous appearance throughout.

At 22 days. During the last day of fetal life the beta cells continue to differentiate as the peripheral reactive cell

masses decrease in size (fig. 7). Intimate contact between the tubules and the peripheral reactive cell masses is maintained, though on the basis of phosphatase activity and cell arrangement, this distinction between the tubule cells and the peripheral reactive islet cells at the point of contact is more easily made. The intense phosphatase activity is no longer continuous into the cells of the tubule wall and the nuclei of the tubule cells are arranged tangentially, as opposed to the radial arrangement seen when the tubules are actively contributing to the islet. Thinning of the peripheral collar of reactive cells and separation of the islets from the tubules becomes much more definite during the first postnatal day. The remaining peripherally located phosphatase reactive cells in the islet at this time represent undifferentiated beta and alpha cells.

Part II. Postnatal development

Continued formation of islets from the intralobular and secondary duct systems occurs during early postnatal life. The series of phosphatase changes described for fetal stages are duplicated during postnatal islet development. Figure 10 demonstrates islet formation from an intralobular duct in a 5 day old rat. The periphery of the islet and the tubule are in continuity by way of a mass of compact $5 +$ cells. The central mass of beta cells exhibits minimal cytoplasmic and only moderate diffuse nuclear activity. A zone of transition is seen between the clear central beta cells and the peripheral collar of reactive cells. Figures 8 and 9 demonstrate islet formation from a portion of the secondary duct system. Here again the minimally reactive beta cells differentiate from the compact masses of reactive cells.

Postnatal islet formation begins during the first or second day, reaches a peak by the end of the first week, and then decreases markedly by the third week. During the second week the peritubular connective tissue begins to show marked phosphatase activity as it becomes highly organized. This organization tends to separate the islets from their tubules

of origin. The fully differentiated islet as seen 20 days postnatally is shown in figure 11.

It has already been pointed out that a striking difference exists between the differentiated beta cells and the cells which compose the parent mass of intensely reactive cells. The differentiated alpha cell, however, exhibits a 4+ activity, and consequently, the exact time at which this cell type can be distinguished from the parent mass of reactive cells is difficult to determine. However, certain cells located in the peripheral collar of reactive cells farthest from the point of attachment of the reactive cell group to the tubule exhibit a slight decrease in cytoplasmic activity and an increase in cell size. In addition, a sharp boundary is seen between these cells and the adjacent beta cells in contrast to the transition in reactivity which is seen at the point of addition of beta cells to the islet by the reactive cell mass.

One striking difference between postnatal and prenatal endocrine differentiation is the tempo at which the changes proceed. It is difficult to locate masses of 5+ cells of any considerable size during postnatal life in which there are no beta cells present. It would seem that before the mass of 5+ cells can attain much size, some of its component cells proceed to differentiate into beta cells. This is quite contrary to prenatal development when large 5+ cell masses retain their activity from the 12th to the 18th day before the beta cells are expressed. Thus, the tempo of beta cell differentiation is seemingly accelerated during late prenatal and postnatal life.

DISCUSSION

The identification of at least two cell types in the adult pancreatic islet on the basis of their phosphatase activity has been reported by Gomori ('41), Jacoby ('46), and others. That the peripheral 4+ reactive cells are the alpha cells seems beyond question because of their constant location on the periphery of the islet, which corresponds to the location of the alpha cells as shown by conventional granule stains

(Gomori, '39b; Hard, '44). Interestingly enough, the difference in reactivity is not so great between the alpha and beta cells in other species, such as monkey and man, in which the two cell types are scattered randomly throughout the islet (Dempsey, Greep, and Deane, '49).

The establishment of adult phosphatase staining characteristics of islet cells during embryonic development has received little attention. Moog ('44) has applied the histochemical phosphatase technique to the chick embryo, but she makes no mention of the endocrine pancreas.

As described, the dorsal pancreatic bud at 12 days exhibits two cell types, one phosphatase rich, and the other relatively phosphatase poor. These phosphatase rich cells in the 12 day bud evidently are not detectable by either hematoxylin-eosin or heidenhain-azan staining, since divergent cell types are not identifiable with these techniques until the 13th day (Hard, '44). Hard's description of the peripherally located nests of differentially staining cells in the 13 day pancreatic cord agrees exactly with our observations of peripherally placed nests of phosphatase rich cells at the same stage of development. Hard identifies his differentially staining cells as primitive endocrine cells. The close correspondence between the developmental histories of the endocrine rat pancreas as shown by conventional staining techniques and of the phosphatase rich cells described here leaves little doubt concerning the identification of the reactive cells as endocrine cells. In addition, the distribution of the phosphatase rich cells, numerous in the dorsal anlage and scarce in the ventral anlage, corresponds to the already established concepts of islet tissue development.

The presence of phosphatase activity in the cells of the dorsal bud which are destined to be endocrine cells serves to emphasize the point that certain changes in the internal chemical composition of prospective divergent cell types occurs before conventional morphological and staining criteria of differentiation give any indication of change. Subsequent generations of endocrine cells, whether from the primitive

cord, primitive tubules, and ducts of the fetal pancreas, or from the intralobular ducts and secondary duct systems of the gland postnatally, exhibit a common pattern of differentiation with respect to phosphatase activity.

Concerning beta cell differentiation, the time at which these cells become recognizable on the basis of similarity to the adult cells in morphological and phosphatase characteristics coincides with the time of appearance of beta granules in the central islet cells as reported by Hard ('44). Thus, the endpoint of differentiation is marked by a decrease in phosphatase activity.

Observations concerning the phosphatase-granulation relationships of the alpha cell are more difficult to make because of the similarity between the phosphatase reaction of the adult alpha cell and its precursor cell.

Concerning the rate of endocrine cell differentiation, the complete expression of the beta cell does not proceed on a rigid time basis, but progresses at a rate which varies, depending on the age of the fetus. This variation in tempo of differentiation is indicated by the varying duration of the period in which the 5 + cell "rests" prior to its expression as a beta cell. At 19 days the total number of foci of beta cell differentiation far exceeds the number of 5 + cell groups derived from the primitive cord. Consequently, some of these foci of beta cell differentiation must represent foci of tubulo-insular cell transformation initiated after the 15th day. For these cells, a period of no more than 4 days is required for expression as beta cells. On the other hand, a period of 5 to 6 days elapses before the beta cells differentiate from the 5 + cell groups derived from the primitive cord. Most of the cells which are rapidly added to the islets from the tubules during the 20th day are differentiated as beta cells by the end of gestation, a period of two to three days elapsing here. Comment has previously been made upon the extremely brief period of 5 + activity in which the potential beta cell "rests" prior to its loss of enzyme activity during postnatal life. Thus, the rate of differentiation increases with

advancing age of the fetus. This is probably a function of the changing humoral (possibly hormonal) conditions of the entire embryo or young animal as development proceeds, a factor which is extrinsic to the endocrine cell itself.

In summary, the salient features of the phosphatase changes which occur during the differentiation of the endocrine pancreatic cells are as follows: (1) The initial phase of differentiation sees the gradual accumulation of phosphatase content within the cell, which at its peak characterizes the parent cell of both the alpha and beta cell types. (2) Subsequently, there is a decrease in histochemically detectable phosphatase which signals the establishment of the functional cell. (3) The rapidity of differentiation increases with increasing age of the animal.

These results as they stand alone are difficult to interpret. However, in combination with observations of alkaline phosphatase in other portions of the developing embryo and with other lines of experimental work, certain workable concepts become evident. The significance of the phosphatase in the developing tissue must be viewed from two different perspectives, namely, in terms of its relationship to fundamental biological processes such as differentiation and proliferation, and in terms of its chemical function within the cell. Complete separation of the embryological processes from the chemical activity of the cell is impossible because of the dependence of the embryological processes upon that chemical activity. Ultimate correlation of these two perspectives is the goal of modern chemical embryology.

In reviewing the localization, concentration, and time of appearance of phosphatase during the development of the endocrine pancreatic tissue it is apparent that different degrees of phosphatase activity characterize different stages of development. Five plus activity characterizes the period of differentiation. That this intense phosphatase activity is associated with the differentiative phase rather than with the proliferative phase is suggested by the following observations: (1) Such maximal 5 + activity may be seen in a single tubule cell. (2) It

is not found beyond the minimal amount in the cells from which the endocrine cells originally differentiate. (3) It sharply diminishes when the cells assume their adult function. (4) No great increase in activity is seen in the central beta cell masses at a time when, according to Hard ('44), many mitotic figures are present. In addition to these observations, Morse and Greep ('47) noted that in the developing tooth epithelium the site of most rapid proliferation is the site of minimal phosphatase activity. In the developing chick spinal cord, the site of maximal proliferative activity is in the alar plate (Hamburger, '48), whereas the site of maximal phosphatase activity is not in the alar plate but in a cross band through the dorsal part of the basal plate (Moog, '43).

The total amount of phosphatase seen in the endocrine cells during their differentiation, however, can not be viewed as being concerned with the differentiative mechanism alone. Undoubtedly, a certain small portion of the enzyme takes part in the process of proliferation, as Willmer ('42) has shown by tissue culture experiments that mitosis is accompanied by a small increase in both nuclear and cytoplasmic phosphatase content. The magnitude of this increase which accompanies proliferation, however, must certainly be much less than that which occurs in the developing endocrine pancreas, since rapidly proliferating tissues in the embryo (heart, skeletal muscle) exhibit only minimal activity with the technique employed in this study.

Studies on certain neoplastic tissues also bear out the relationships between phosphatase activity and the processes of differentiation and proliferation. Greenstein ('43) states that the activity of alkaline phosphatase is unchanged or even reduced slightly with the onset of the neoplastic state, though there are notable exceptions to this statement. Atkinson and Gusberg ('48) report that rapidly proliferating neoplasms of the uterine epithelium possess no phosphatase except in the capillaries of the blood vessels. The more differentiated carcinomas of the uterine epithelium possess more phosphatase than the highly malignant types, but less than the epithelium

of simple benign cysts. Thus, the bulk of the evidence indicates that simple proliferation of cells is not dependent upon high concentrations of alkaline phosphatase, and that intense phosphatase activity seen in certain tissues, in this case the endocrine pancreas, is definitely associated in point of time with the differentiative phase of the cells.

The exact biochemical significance of alkaline phosphatase in the developing endocrine cells is difficult to determine because of our incomplete knowledge of the chemistry of differentiation and proliferation. A complete discussion of this subject must include the problems of substrate supply for the anabolic and catabolic reactions, substrate transfer, energy mechanisms, and finally, the mechanisms which are responsible for the unidirectional alterations of the protein and enzyme chemistry of the cell which occur as it differentiates and assumes its characteristic chemical nature. The picture is further complicated by the fact that within each cell a portion of the contained enzyme may be concerned with one of the above processes, a portion of it with another, and so on.

That the large amount of phosphatase seen in the developing endocrine pancreatic cells plays a part in the breakdown of certain substrates with the liberation of energy necessary for cell maintenance and synthetic activity seems quite tenable in the light of our present knowledge concerning the importance of the phosphate radical in these processes. However, in view of the fact that all tissues do not progress through a phosphatase rich differentiative phase during their differentiation, this suggestion seems unlikely, unless there is a difference in the type of substrate which the different tissues are metabolizing. Present information sheds little light upon this aspect of the problem.

That a certain portion of the phosphatase plays an important role in the transfer of substances across membranes and molecular films would seem to be indicated. Wislocki and Dempsey ('45) suggest that an important phosphatase barrier exists between sites of glucose supply and glycogen deposition. The presence of large amount of phosphatase in the adult

duodenal epithelium and brush borders of the proximal convoluted tubules of the kidney as shown by numerous investigators also supports this thesis. Baud and Fulleringer ('48) report that phosphatase, among other enzymes, is found in the nuclear membrane. Undoubtedly, a certain portion of the enzyme encountered in the present study is important to the transfer of substances across membranes.

That most of the phosphatase seen in the endocrine cells here described is a part of the mechanism of differentiation would seem to be indicated by its close association with the differentiative phase of the cells. In this connection, a growing mass of evidence indicates a specific relationship between the alkaline phosphatase and the nucleoproteins of the cell. Krugelis ('46) reports the existence of three different intracellular phosphatase systems. One is a monophosphatase which is present in both the nucleus and the cytoplasm when mononucleotides and glycerophosphate are used as substrates. The other two are diesterases which apparently are located either in the nucleus or in the cytoplasm, depending on whether or not depolymerized desoxyribonucleic acid or ribonucleic acid respectively is used as substrate. Nuclear alkaline phosphatase in the chromosomes of the salivary gland cells of *Drosophila* is concentrated in the euchromatic bands, which are known to represent the location of desoxyribonucleoproteins (Danielli and Catcheside, '45; Krugelis, '46). Brachet and Jeener ('47) report a close parallelism between the intensity of alkaline phosphatase activity of the cell nuclei of vertebrate tissues and the rate of labeled phosphorus (P^{32}) turnover, which in turn involves nuclear desoxyribonucleic acid. Jeener ('46) reports a chemical association between thymonucleoprotein and alkaline phosphatase as a result of his studies. Other histochemical research also indicates that the degree of cytoplasmic basophilia, chiefly due to the presence of ribonucleoproteins, is correlated with the concentration of cytoplasmic alkaline phosphatase (Abolins, '48; Dempsey and Wislocki, '46). Certain other studies, however, indicate an inverse relationship between these two sub-

stances (Wislocki and Dempsey, '45; Bodian and Mellors, '45). This apparent discrepancy must await further work for complete resolution. In any event it seems that there is a distinct association between the phosphatases and the nucleoproteins which is of some functional significance.

The significance of this association between the nucleoproteins is difficult to ascertain, though several important possibilities present themselves. Distinct association has been established between the gene, enzyme, and protein components of the cell. Desoxyribonucleoprotein is the chief constituent of the gene. Ribonucleoproteins are apparently associated with protein synthesis. Furthermore, the intracellular enzymes, under direct control of the component genes of the cell, are responsible for protein synthesis. The exact position of alkaline phosphatase within this entire scheme is still questionable.

It would seem that the large amount of phosphatase seen in the developing endocrine cells might be associated with the gene-enzyme relationship. It is this gene-enzyme relationship which essentially constitutes the process of differentiation, the process by which certain characteristic enzymes, under genic direction, gain ascendancy in the molecular population of the cell and thus establish the differentiated character of that cell. Unpublished observations on other developing tissues in the embryo, however, indicate that this great increase in phosphatase is not enjoyed by all differentiating tissues. Before this can be stated definitely, more critical quantitative analyses of the phosphatase changes in these apparently refractive tissues must be made. The possibility that all tissues do not pass through a phosphatase rich differentiative phase would not seem too unlikely. It would hardly be expected that a single common differentiative pathway would suffice for the multiplicity of terminally differentiated cells in the animal organism.

Recent interesting studies by Atkinson and co-workers ('49) have a direct bearing on the above mentioned gene-enzyme-protein scheme. According to these workers rapidly prolifer-

ating endometrial carcinomas and cells in the proliferative phase of the menstrual cycle exhibit an increase in cytoplasmic basophilia, presumably due to increased amounts of ribonucleic acid. However, in the carcinomatous cells, there is little or no phosphatase present (Atkinson and Gusberg, '48); whereas in the normal proliferative phase, there is an increase in phosphatase (Atkinson and Engel, '47). Thus, in the carcinomatous cells, rapid synthesis of proteins is occurring even though there has occurred no increase in stainable phosphatase. In the normal proliferative phase, however, there is a marked increase in phosphatase activity. This difference may well be an expression of the differentiation of the normal proliferating cells into secretory cells with termination of proliferative activity, precisely the property which the carcinomatous cells do not possess. It is as if the carcinomatous cells have lost their genic control of the enzymes of the cell, resulting in continuous proliferation with no differentiation. Herein may lie the association of the phosphatase system with the genes or nucleoproteins and the enzymes. In conclusion, there is a great increase in alkaline phosphatase in the developing endocrine pancreatic cells during their differentiation. This increased quantity of enzyme appears most apt to be associated with the mechanisms by which the genic components of the cells exert their influence in determining the enzymes of the differentiating cell. However, at any one time during the development of these cells a certain small amount of the cellularly contained phosphatase is involved with other chemical functions, chief of which is probably the transfer of substrate materials across membranes and molecular films.

SUMMARY

1. The concentration of alkaline phosphatase within the differentiating endocrine pancreatic cell increases from a minimal activity to a 5 plus reaction at which time it decreases sharply to the degree of enzyme activity characteristic of the adult functional cell.

2. Both the alpha and beta cell types exhibit this sequence of changes, though the disappearance of the phosphatase from the beta cells is much more complete than from the alpha cell.

3. The rate of differentiation increases with advancing age of the fetus, being more rapid in late fetal life and early postnatal life than in early fetal life.

4. The high concentration of phosphatase is characteristic of the differentiative phase rather than of the proliferative phase.

5. It is suggested that possibly the large amount of phosphatase present during the differentiative phase plays some role in the interaction between the gene and enzyme.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Dorsal pancreatic bud attached to foregut in a 12 day embryo. The black cells in the bud are the phosphatase rich endocrine cells. Along the left side of the photograph are the phosphatase rich neural tube and the dorsal aorta. Erythrosin counterstain. $\times 100$.
- 2 Dorsal pancreatic lobe of 13 day embryo. Primitive endocrine cells in surface of anlage. Erythrosin counterstain. $\times 100$.
- 3 Dorsal pancreatic lobe of 14 day embryo. Peripheral growth of exocrine cords and invasion by mesenchyme have left the endocrine cells centrally located. Erythrosin counterstain. $\times 100$.
- 4 Central masses of endocrine cells and their tubules of origin in 16 day fetal pancreas. Masses of phosphatase rich endocrine cells bulge from the tubule walls, T. No counterstain. $\times 500$.

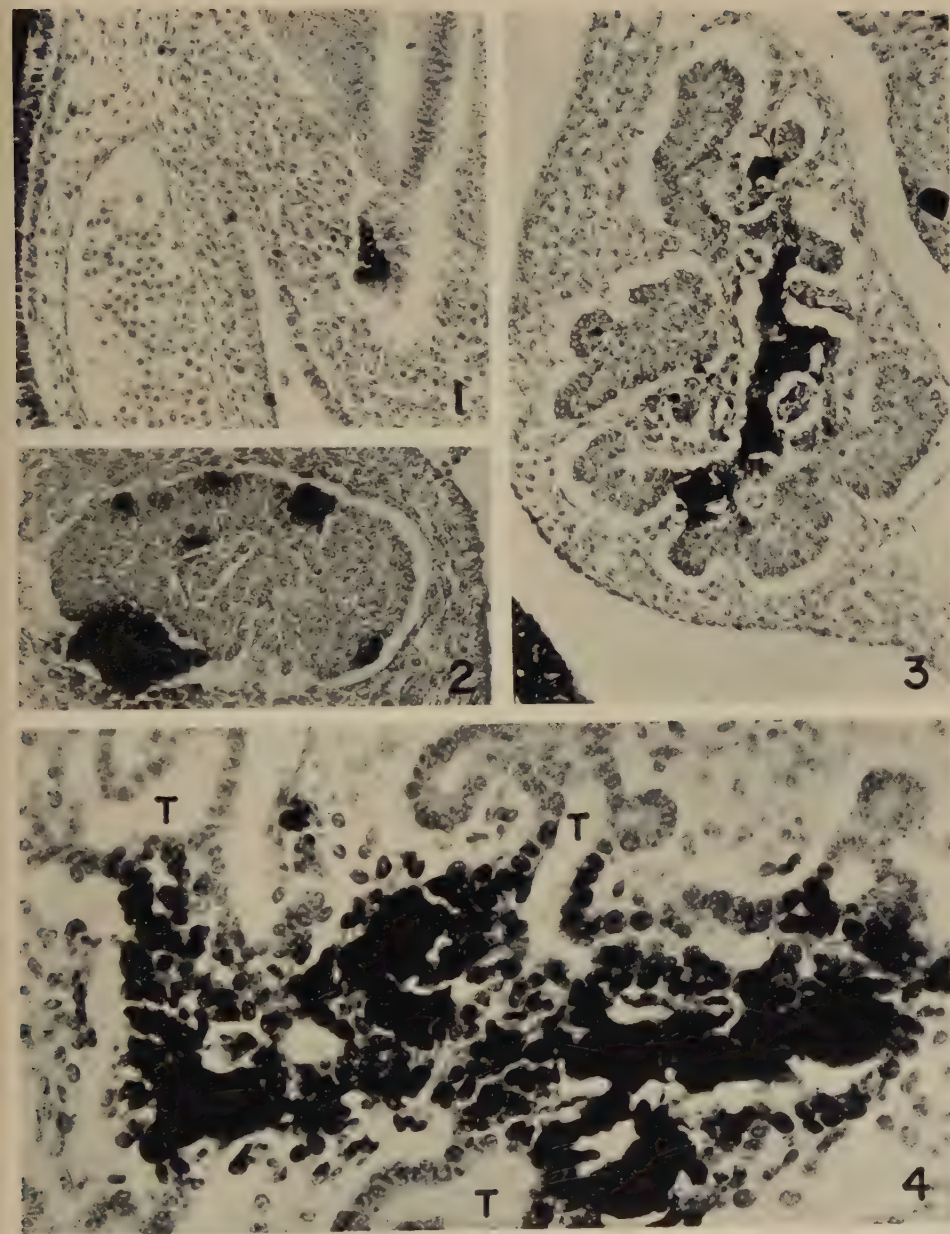


PLATE 2

EXPLANATION OF FIGURES

5 Mass of phosphatase rich cells forming a bridge between adjacent tubules, T, of 18 day fetal pancreas. The apparent density of the acinar and duct epithelium is due to the counterstain rather than to sulfide precipitate. Newly differentiated beta cells (arrow) are present. The capillaries invading this mass of beta cells are not seen in this section. Azocarmine — fast green counterstain. $\times 500$.

6 Islet complexes from 20 day old fetus. Density of acinar and duct epithelium due to counterstain. The beta cells (arrows) are differentiating from the phosphatase rich "bridges" of cells attached to the tubules. Note the layer of mesenchyme between the tubule walls and the masses of beta cells. Azocarmine — fast green counterstain. $\times 500$.

7 Islets from 22 day fetus closely associated with tubule. Note single phosphatase rich cell in tubule wall. Cords of beta cells radiate from the center of the islet to the peripheral collar of phosphatase rich cells. Eosin counterstain. $\times 250$.

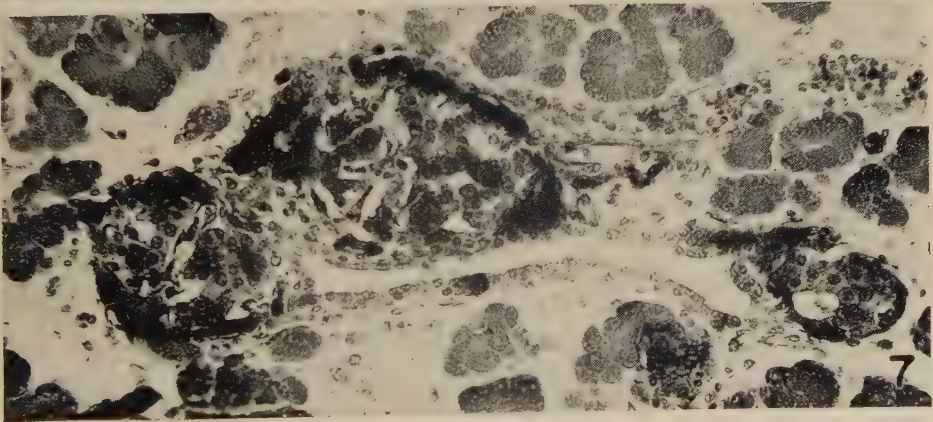
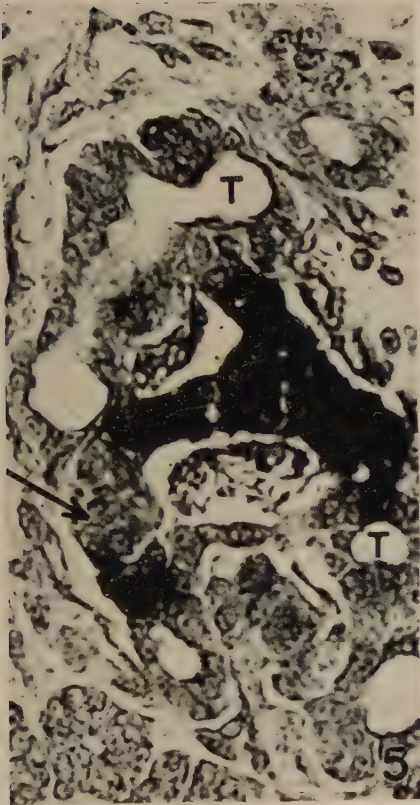


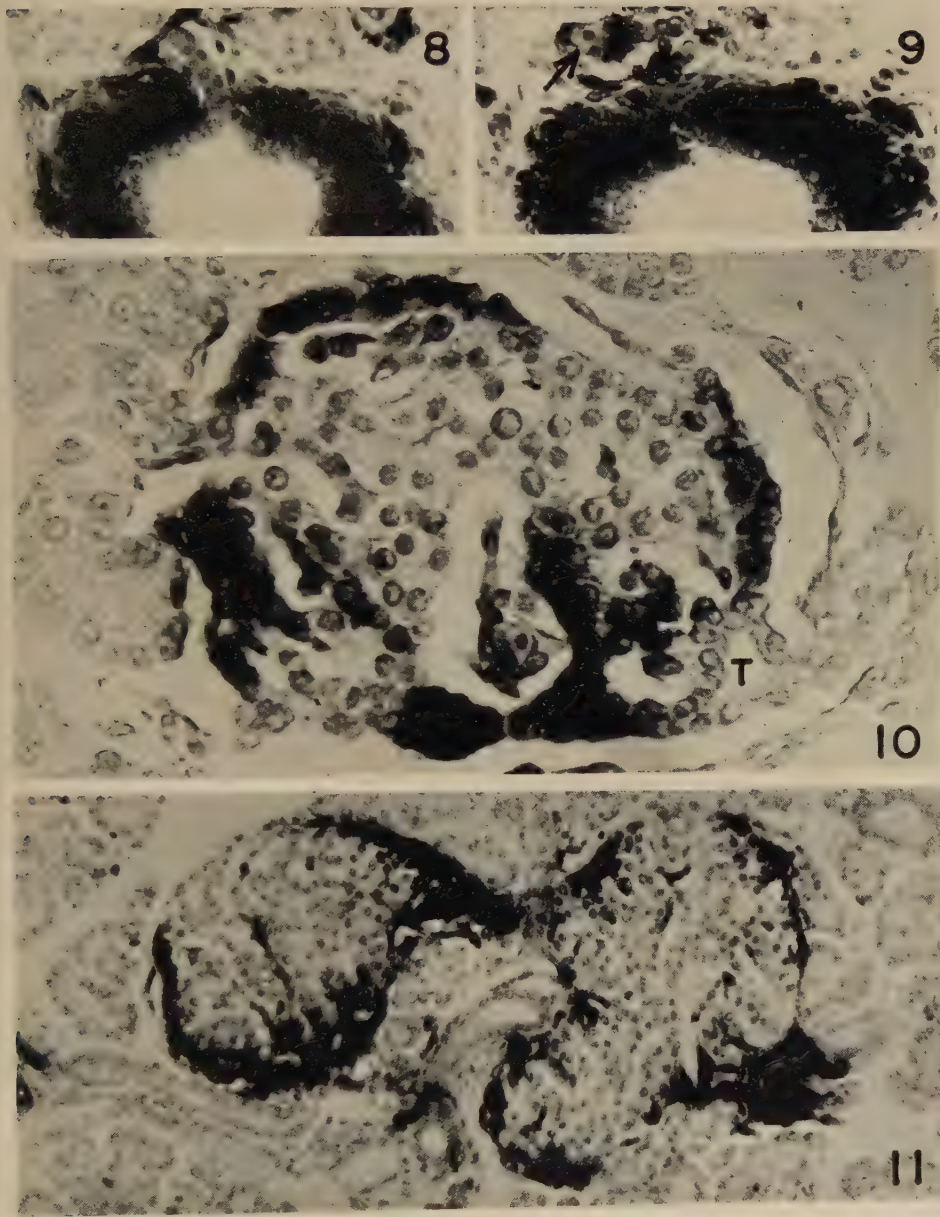
PLATE 3

EXPLANATION OF FIGURES

8-9 Figure 8 shows a cord of cells (secondary duct system) continuous with duct epithelium in 15 day postnatal pancreas. Figure 9 shows the continuity of this same cord with a small mass of phosphatase rich cells in which two beta cells (arrow) are seen. No counterstain. $\times 300$.

10 Postnatal islet formation in 5 day old rat. Continuity between tubule, peripheral phosphatase rich cells, and beta cell shown clearly. Tubule indicated by T. No counterstain. $\times 500$.

11 Islet of 20 day old rat. Central mass of beta cells surrounded by a peripheral collar of phosphatase rich cells. No counterstain. $\times 250$.



THE HISTOGENESIS AND HISTOLOGY OF AN INTEGUMENTARY TYPE OF EPITHELIUM IN THE HUMAN HYPOPHYSIS¹

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TEN FIGURES

Luschka (1860) described areas of epithelium in the capsule of the human hypophysis that resembled the epithelium of the oral mucosa. Similar epithelium has been identified in the pars tuberalis, and nearby areas, by Erdheim ('04 and '26), Guizzetti ('13 and '25), Christeller ('14), Kiyono ('24 and '26) and Susman ('32). Shanklin ('46 and '48) differentiated this epithelium, which is usually characterized by the presence of intercellular bridges, from the arachnoidal mesothelium, which lacks bridges. Guizzetti ('13 and '25) described the stages of differentiation of these cells in what he called prepavement epithelial cords and referred to the differentiated cells as pavement epithelium. Romeis ('40) included in his volume on the hypophysis a discussion of this epithelium based largely on the work of Guizzetti.

It is the purpose of this paper to describe the histogenesis and histology of an integumentary type, or stratified squamous epithelium, observed in our series of 100 human hypophyses.

¹ This investigation was supported by a grant from the Committee for Research in Endocrinology, National Research Council.

² The writer wishes to thank Mr. Tamir Nassar, Technician, for assistance in preparation of the material and Mr. Haigaz Artinian, Photographer, for making the photomicrographs.

OBSERVATIONS

Incidence. Stratified squamous epithelium was found in hypophysis M91 from a 40-year old male whose death was due to suicide by hanging, M115 was from a 40-year old male who died from progressive paralysis, M217 was also from a 40-year old male whose death was due to suicide by poison, M224 was from a 52-year old female who died from a brain tumor, M229 was a 23-year old male whose death was due to a brain tumor, and M233 was an 86-year old male who was killed by a brain hemorrhage.

For a summary of the histological findings the reader is referred to the paper by Shanklin ('48, table 1).

Histogenesis and histology. Hypophysis M229 (figs. 1 and 2) included in the anterior capsule of the lower stalk areas of epithelium illustrating the stages including poorly differentiated cells to mature stratified squamous epithelium. Four of these areas were 27×36 , 37×71 , 58×65 and $73 \times 110 \mu$ respectively on cross section. The smaller areas were surrounded by well developed basement membranes. The smallest island of poorly differentiated cells included 10 closely packed oval nuclei surrounded by scanty cytoplasm, without visible cell membranes. Two other areas had peripheral zones of one to three cells wide surrounding a central area of 7 to 10 cells. The cells of the outer zone were flattened and had concentrically arranged nuclei surrounded by scanty cytoplasm. These peripherally placed cells were similar to those in the smallest area. The nuclei of the central cells were slightly larger, oval to round, but essentially the same as the others. The cytoplasm was abundant, finely granular and slightly eosinophilic. The cell membranes were very distinct and markedly eosinophilic (fig. 1 c).

The largest area, seen in three parts (fig. 2), were joined together inferiorly. Some of the nuclei of these cells were nearly naked while others were surrounded by abundant cytoplasm. A few cells were hydropic. Intercellular bridges were found in the large area, but not in the smaller ones.

The open spaces in the areas, the presence of pyknotic and naked nuclei and the extensive cytoplasmic fragmentation cannot be attributed to post-mortem changes but appear to represent early cyst formation.

The stratified squamous epithelium in hypophysis M91 consisted of numerous round or oval areas (figs. 5 and 6) situated in the capsule of the anterior aspect of the stalk and in the substance of the pars tuberalis. The main part formed a large triangular area, with the base at the level of the attachment of the stalk to the infundibular process and with the apex extended upward. The uppermost areas consisted of long, thin cords of cells in the capsule. None of this epithelium was observed in the adjacent neurohypophysis.

The larger areas were surrounded by poorly developed capsules, but the long thin areas lacked capsules. Highly developed basement membranes were well stained by the Mallory method. The smallest areas included irregularly scattered medium-sized oval to round nuclei surrounded by scanty lightly stained cytoplasm. The nuclear membranes were well stained and the relatively small chromatin granules were moderately stained. There were no nucleoli. Most of the cells in the smaller areas were poorly differentiated, but many of those in the larger areas were mature stratified squamous cells.

The larger areas had a peripheral zone of two to three layers of poorly differentiated cells, similar to those in the smaller areas, which were often concentrically arranged, surrounding a central area of larger cells. The line of demarcation between the two areas was often very sharp. Some of the larger cells were also concentrically arranged. The central mature cells had abundant granular cytoplasm and highly developed cell membranes. Their cytoplasm was stained light blue after Mallory while the cytoplasm of the peripheral cells was stained very light reddish-brown. Distinct tonofibrils and beautiful intercellular bridges were present in the central cells, but none were observed in the peripheral layer. Because of their histological appearance we believe

that the cells of the outer zone are comparable to those of the stratum basalis of the skin while those of the central area are similar to those of the stratum spinosum. There was an occasional pyknotic nucleus in the spinous layer of some areas. The central part of several areas had large cells with granular lightly stained fragmented cytoplasm. The nuclei in the central areas were considerably larger than those at the periphery, but were otherwise similar.

There was a single area of stratified squamous epithelium measuring $55 \times 140 \times 146 \mu$ in specimen M115 (fig. 3). It was situated in the capsule of the anterior surface of the lower part of the stalk. It had no differentiated capsule, but a well developed basement membrane was present. The epithelium consisted mostly of spinous cells. The chromatin of the nuclei was darkly stained, and the granules were very irregular in shape, size and distribution. Some of these cells had nucleoli. The cytoplasm of these cells was abundant and finely granular. Some of these cells had prominent intercellular bridges.

The stratified squamous epithelium in hypophysis M217 (figs. 7 and 8) consisted of multiple areas situated in the capsule on the anterior surface of the stalk. These areas consisted of three groups: upper, middle and at the transition zone. The upper group consisted of many long thin areas, the middle group of somewhat broader long areas and the lower group of oval elongated areas. Measurements of representative areas in one section were, upper 35×290 , middle 95×1070 and lower $160 \times 598 \mu$. The larger areas were surrounded by poorly developed capsules containing a few long flattened fibroblast nuclei. The basement membranes were well developed. The smaller areas consisted of an outer zone of smaller cells surrounding a central area of much larger ones (fig. 7). The larger areas (fig. 8) were characterized by large round to oval cells with large round to oval well stained nuclei. These cells had abundant cytoplasm which contained scattered eosinophilic granular material. They were surrounded by strongly eosinophilic cell membranes. A few of

the cells were nearly empty. Although the cytoplasm of these cells appeared to be breaking down, most of the nuclei were normal.

A single area of stratified squamous epithelium $98 \times 143 \times 280 \mu$ was situated among the loosely arranged collagenous fibers of the upper aspect of the anterior lobe capsule of pituitary M224 (figs. 9 and 10). This area was not organized into basal and spinous layers; in some sections, however, the central area was sharply separated from the outer part for the peripheral cells were more flattened than the central ones (fig. 10). The nuclei were of average size with rather small irregularly arranged chromatin granules. There were no nucleoli. Most of the cells were separated from one another by intercellular spaces traversed by well developed intercellular bridges. Their cytoplasm was filled with very fine slightly eosinophilic granular material.

The single area of stratified squamous epithelium in specimen M233 (fig. 4) measured $50 \times 77 \times 153 \mu$. It was situated among loosely arranged collagenous fibers in the capsule of the pars anterior near the attachment of the stalk. A highly developed strongly eosinophilic basement membrane encircled the area. The epithelium was differentiated into a peripheral layer of cuboidal and angular cells and a larger central part which included a round body of concentrically arranged cells. Some of the nuclei in both parts were lightly stained while others were very darkly stained. Well developed nucleoli were present. The cytoplasm of the peripheral cells was scanty but that of the central cells was abundant and finely granular. Intercellular bridges were present. The area of concentrically arranged cells included several small, darkly stained pyknotic nuclei embedded in very strongly eosinophilic material. This material was separated into layers by highly refractive bands, which represented the previous cell boundaries. The nuclei and cytoplasm of the adjacent cells were flattened and arranged concentrically around the pinkish mass. The nuclei of these cells had well stained membranes but most of their chromatin had disappeared and they appeared to be degenerat-

ing. This rounded body resembled a Hassall's corpuscle, characteristic of the thymus.

DISCUSSION

Only 6 (6.0%) of the 100 hypophyses had stratified squamous epithelium with intercellular bridges. These were from individuals varying from 23 to 86 years old. Erdheim ('04) found islets of epithelium in 10 out of 13 hypophyses. He failed to find similar cells in 7 foetal and new-born specimens, although he thought they were present but were not observed due to their small size. Christeller ('14) found them in 20 out of 34 cases. They varied from a single small islet to a mass in one case which completely replaced the pars anterior. Kiyono ('24) found islets of epithelium in 17 out of 50 hypophyses. All were 20 years or older. We believe that the above writers failed to differentiate between islets of mesothelium, derived from the meninges and stratified squamous epithelium, originating from Rathke's pouch material; therefore, it is impossible to determine from their papers the incidence of each variety. All three investigators referred to the presence of intercellular bridges, hence it is clear that some of them were stratified squamous epithelium. Susman ('32) found "squamous rests," with intercellular bridges, in 32 out of 230 specimens. The highest incidence was between 40 and 49 years.

The findings in our series and that of Susman indicate that the incidence is from 6.0 to 13.9% with the maximum number occurring in the 40- to 49-year old group. Five of our 6 positive specimens were from males. Susman failed to include the sex in his series.

The cords of poorly differentiated epithelium described by Guizzetti ('13 and '25) in the stalk included smaller areas with nuclei surrounded by sparse cytoplasm and larger areas showing varying degrees of differentiation. Our findings are in accord with those of Guizzetti.

The least differentiated of these areas of epithelium consisted of 5 to 10 cells with medium sized nuclei and very

scanty cytoplasm. In the next stage the central cells had more cytoplasm and were stained more lightly, but there was no sharp line of demarkation between central and peripheral areas. In the next stage the central cells had abundant finely granular cytoplasm and distinct cell membranes. The nuclei were round to oval and larger than the peripheral ones which were frequently flattened and concentrically arranged. The nuclei, like the cytoplasm of the central cells, were often more lightly stained than the peripheral ones. This stage is the precursor of the areas whose cells can be differentiated into basal and spinous-celled layers. Guizzetti ('13 and '25) and Kiyono ('24) noted that the smaller islets were often of the basal cell type and that the cells of the larger islets were like the prickle-cells in the stratum spinosum. Our findings were similar to those of Guizzetti and Kiyono. Erdheim ('04) gave the first accurate description of these epithelial islets and described peripherally placed basal cells and centrally placed spinous cells both in the islets and in the tumors derived from them.

The central cells showed considerable variation in the different specimens. All had fairly abundant cytoplasm, often with well developed cell membranes and with beautiful intercellular bridges. In many islets the cytoplasm of an occasional cell was fragmented; in some many of the cells had very granular fragmented cytoplasm, while in others the central area showed early stages of cavitation (fig. 2).

Erdheim ('04) established the relationship between these islets of stratified squamous epithelium and the hypophyseal stalk cysts. He described (p. 575) one specimen from an 86-year old male where the basal cells were high cylindrical but the central part showed swelling and softening with a small fluid filled cyst (fig. 2 h). Kiyono ('24) interprets the small cavity with fragmented cells in his figure 4 as the beginning of cyst formation. We believe that the small cavities described above in our material also represent early cyst formation.

Most of Rathke's pouch material forms the hypophyseal vesicle, but some cells lag behind as the hypophyseal duct cells. From the vesicle develop the anterior lobe and ciliated columnar cells, which frequently surround the follicular cysts (Shanklin, '49 and '50). The ciliated columnar cells may be identical histologically with those of the trachea (Shanklin, '50, fig. 17). Erdheim ('04) and Duffy ('20) believe that the duct epithelium is different from that of the vesicle. According to Erdheim the duct epithelium is closely related to the oral epithelium and often gives rise to stratified squamous epithelium.

Duffy ('20), Frazier and Alpers ('34) clearly differentiated between hypophyseal cysts derived from Rathke's cleft, or the residual lumen, and those originating from stratified squamous cells in the capsule. The histogenesis and histology of Rathke's cleft cysts have recently been described (Shanklin, '49 and '50).

Erdheim ('04, p. 572) states that the largest areas of stratified squamous epithelium are found in older persons. The most extensive areas in our specimens were in a 40-year old male. Although areas of this epithelium normally remain small in younger individuals it is in this age group that they frequently form tumors (Cushing, '27; Beckmann and Kubie, '29).

We conclude that an integumentary type of epithelium occurs in from 6.0 to 13.9% of human hypophyses and is most frequently observed in the older age groups. This epithelium is derived from the hypophyseal duct epithelium by a series of developmental stages beginning with poorly differentiated cells and ending with mature stratified squamous epithelium with intercellular bridges.

SUMMARY

1. Stratified squamous epithelium was found, chiefly in the capsule of the stalk, in 6 out of 100 human hypophyses.
2. Stages including poorly differentiated cells to mature stratified squamous epithelium were identified and described.

3. The areas of mature epithelium frequently consisted of a peripheral layer of poorly differentiated and a central area of highly differentiated cells with intercellular bridges.

4. Pyknotic nuclei, fragmented cytoplasm and cavitation were observed in some areas suggesting early cyst formation.

5. The role of these cells in the formation of hypophyseal duct tumors is discussed.

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PLATE 1

EXPLANATION OF FIGURES

All photomicrographs are from sections of the human hypophysis stained with hematoxylin and eosin. All magnifications are $\times 425$, except figure 5 which is $\times 130$.

- 1 Section illustrating stages in differentiation of stratified squamous epithelium in capsule of stalk of hypophysis M229.
- 2 Section showing a region of stratified squamous cells illustrating early cyst formation from hypophysis M229.
- 3 Section through capsule of stalk including a single area of stratified squamous epithelium from hypophysis M115.
- 4 Section through capsule of pars anterior of hypophysis M233 including a single area of stratified squamous epithelium.

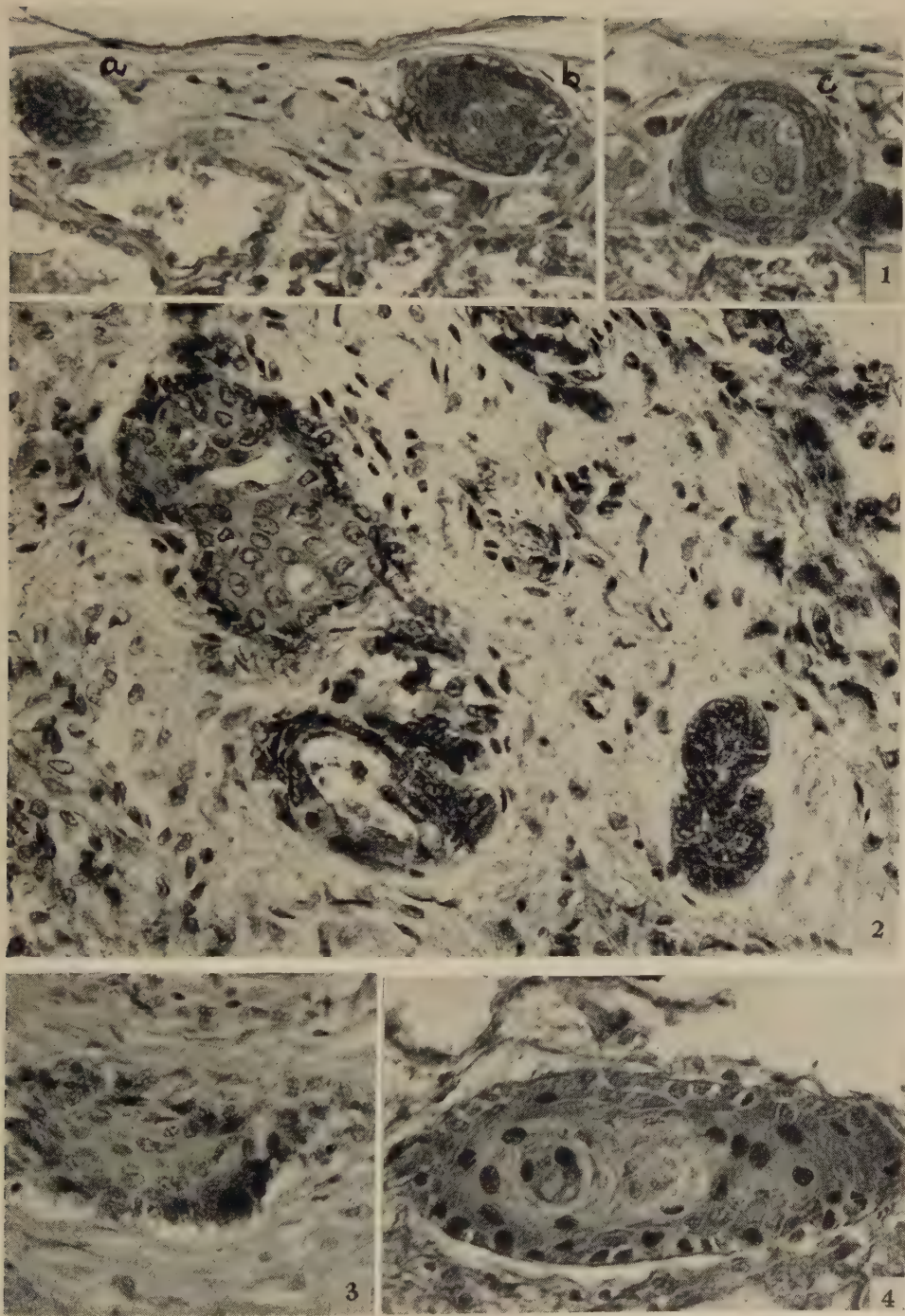


PLATE 2

EXPLANATION OF FIGURES

5 Section illustrating multiple areas of stratified squamous epithelium in hypophysis M91.

6 Some of the above areas more highly magnified.

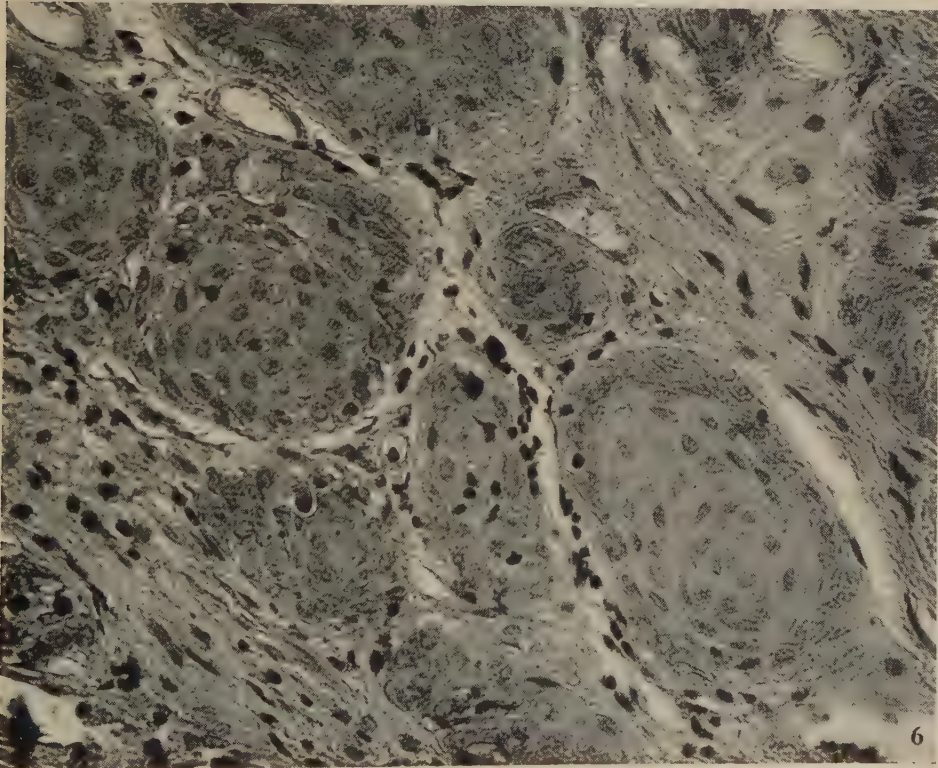
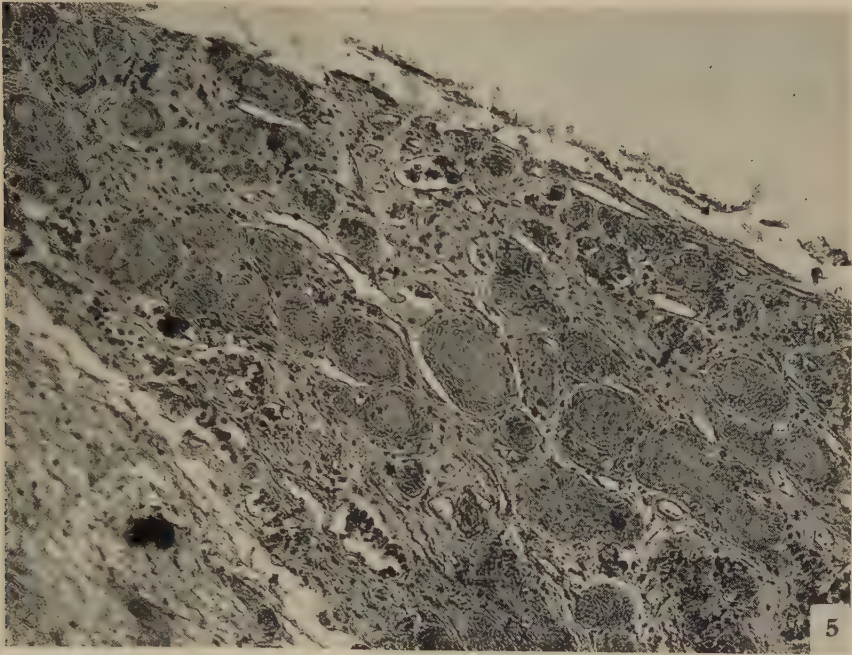
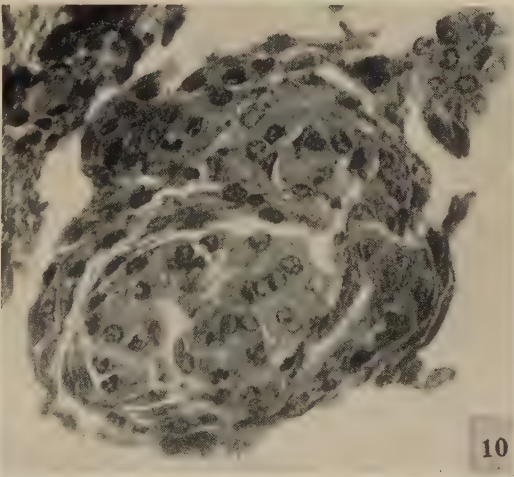
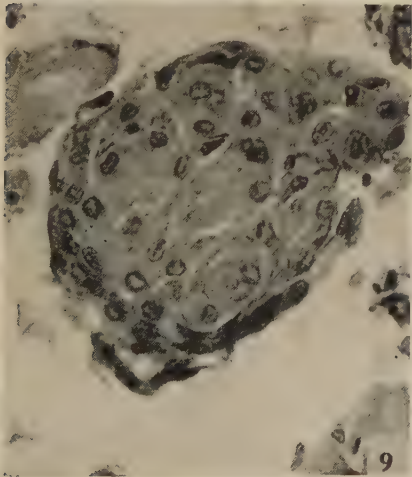
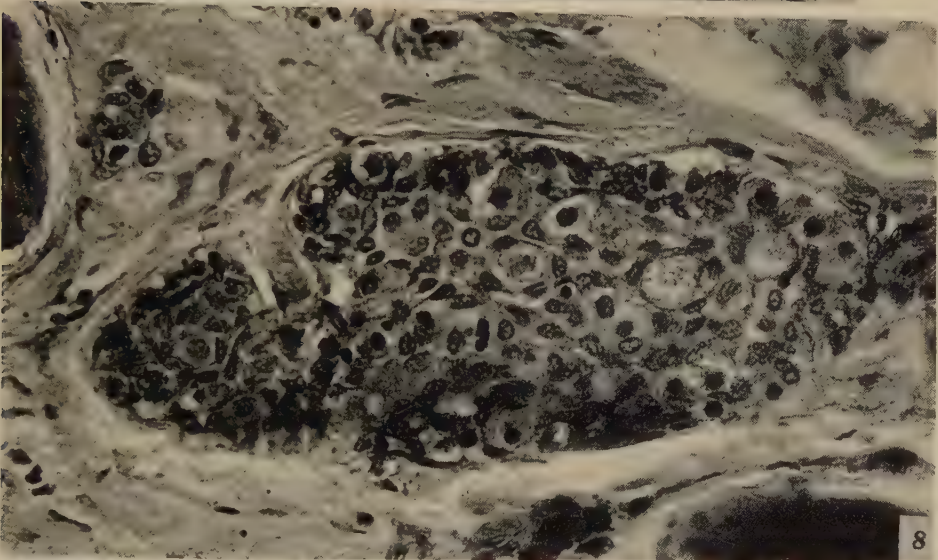
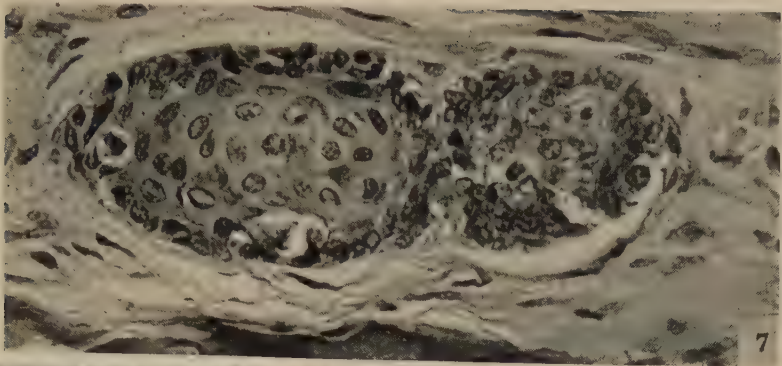


PLATE 3

EXPLANATION OF FIGURES

- 7 Section showing an area of stratified squamous cells in capsule of stalk of hypophysis M217.
- 8 Section from same specimen including area of stratified squamous epithelium showing cytoplasmic fragmentation.
- 9 Section from capsule of anterior lobe including one end of an area of stratified squamous cells in hypophysis M224.
- 10 Central part of same area illustrated in preceding figure.



THE INCIDENCE OF SEPARATE NEURAL ARCH AND COINCIDENT BONE VARIATIONS

A SURVEY OF 4,200 SKELETONS

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THREE FIGURES

Many investigations have been made on skeletonized vertebral columns to establish the incidence of the separate neural arch (spondylolysis, spondyloschisis, cleft pars interarticularis or cleft isthmus), as well as to seek out the nature of its origin (Congdon, '32; Friberg, '39; Hasebe, '12-'13; Hayek, '28; Schwegel, 1859; Shore, '28-'29; Stewart, '31-'32; Willis, '31). However, the methods of determining the frequency of its occurrence in relation to age have raised in some aspects the question of statistical significance and acceptability. For although incidence of the separate neural arch as related to age was previously investigated by Willis ('31) in a portion of the skeletons used for this study, and by Friberg ('39) on roentgenographic material, such observations are here repeated but on this altered basis: computations are made for populations of the same race and sex rather than for a group, including both sexes and different races. This makes an important difference as will be seen later when the incidence will be found to vary significantly with race and sex.

In addition to this, no studies have been found in which the vertebral columns presenting separate neural arches have been examined for the presence or absence of certain specific congenital or acquired characters. Such information — whether or not neural arch separations are more frequently accompanied by congenital defects, arthritis, or other abnormality of the column — may contribute toward a better evalu-

ation of the condition itself, and the spinal column as a whole in those patients who present the separate neural arch.

The authors have had an unusual opportunity to make such a study because of ready access to a large volume of skeletal material, and because the frequency of most of the characters under consideration (number of presacral segments, spina bifidas, etc.) has been established previously for groups of skeletons within these same collections (Lanier, '39; Moore, '24; Todd, '12; Trotter, '37 and '47; Willis, '23-'24).

MATERIAL AND METHODS

The present study was made on the skeletons of the Terry Anatomical Collection of Washington University in St. Louis, and the Todd Collection of Western Reserve University in Cleveland.¹ Both of these collections have remarkable similarities insofar as they are derived from the same relative socioeconomic level, have similar distribution as to age, sex and race, and have been prepared by approximately the same methods. It is appreciated that the purity of the racial stock is open to considerable question especially with regard to the American Negro and that the use of the term (American Negro) no longer can be considered as one implying an unmixed racial strain (Terry, '32). Certainly, but to less extent, the same may be said of the White race. Nevertheless, the material has been used for many previous skeletal studies, (Lanier, '39; Moore, '24; Terry, '32; Todd, '28 and '12; Trotter, '37 and '47; Willis, '31 and '23-'24) and the content of the two collections is generally appreciated. A total of 4,200 skeletons form the basis of this report: of these 1,952 were White males, 1,339 were Negro males, 348 were White females and 561 were Negro females.

This study was confined largely to the lumbar vertebrae, and was extended to the rest of the column only when a

¹ Grateful acknowledgment is made to Dr. N. L. Hoerr, head of the Department of Anatomy, and Dr. W. H. Sassaman, Curator of the Hamann Museum, Western Reserve University, for their permission to study the skeletal material of the Todd Collection.

lumbar vertebra was shown to present a separation through the pars interarticularis of the neural arch. In order to be certain that all lumbar segments were present in each particular specimen, the lumbar vertebrae were carefully articulated and counted, and in those cases in which the sacrum was present the lumbosacral articulation was approximated. No lumbar spine was considered complete unless the articular facets seemed to fit properly throughout. When there were only 4 lumbar vertebrae, the specimen was considered to be unsatisfactory unless the articulations of the lumbar vertebrae fitted satisfactorily with each other, with the lowest thoracic vertebra and with the sacrum. In three cases, the lumbar segment was not complete but these were included because they presented clearcut and unmistakable separations. Either elimination or inclusion of these specimens is open to discussion. However, the difference made in percent by three cases out of a group this large alters the figures very slightly in either direction.

Bilateral separations through the pars interarticularis were readily apparent but detection of unilateral separations required more careful scrutiny and consequently each isthmus was examined individually. All columns having isthmal defects were subjected to additional examination for the following characters: total number of presacral vertebrae, articular facets for cervical ribs on the 7th cervical vertebra, spina bifida, articulations of the transverse processes of the 5th lumbar vertebra with the sacrum or ilium or both, arthritis of the central atlantoepistrophic joint, arthritis of the articular processes of the lumbar vertebrae and certain anatomical variations in the sacrum, including accessory sacroiliac articulations, incomplete fusion of the first sacral segment, spina bifida of upper sacral segments, complete spina bifida or open dorsal bony wall of the sacral canal, and rudimentary sacral articular processes.

The various arthritic manifestations studied were grouped into classifications of mild, moderate and severe. This was done by the arbitrary method of gross examination of the

articular processes for evidence of wear, deformity and spurring. Those cases of arthritis classified as mild presented only slight lipping or spurring of the facets (1 mm or less) with rounded edges and with smooth articular surfaces. Moderate cases presented longer, sharper and more irregular spurs and at times irregularity of the joint surfaces. The severe changes consisted of sharp, irregular spurs at the facet margins, irregularity of the articular surfaces, and frequently erosions at the margins of the facets where the pedicle or lamina adjacent to the articular surface had been impinged upon. In addition to this, those cases classed as severe tended to involve several or all lumbar vertebrae rather than one or two as in the moderate or mild cases. As can be seen there may be some overlap and, thus, chance for error in such a classification. Nevertheless, the result does indicate a trend and should be considered as such.

RESULTS

The results of this study are presented in tabular form for ready reference. Of the 4,200 skeletons accepted for this study 178 had isthmal defects, yielding an over-all incidence of 4.2%. On the basis of race and sex, however, the incidence was 6.4% for White male skeletons, 2.8% for Negro male skeletons, 2.3% for White female skeletons, and 1.1% for Negro female skeletons. It can be seen from table 1 that although there were variations in the percentage incidence for each decade in each group, the only widely divergent figures occurred in those decades where numbers were not sufficiently large to support valid conclusions. Hence, the incidence of neural arch defects remained essentially unchanged within each group from approximately 20 to 80 years of age. Statistical analysis (Chi square) revealed no significant difference in incidence between the groups 49 years of age and under and those 50 years of age and over.

In table 2 is summarized the incidence of certain bone variations in those columns showing a separate neural arch. The number of columns showing neural arch separations was

TABLE 1
Incidence of separate neural arch according to age, sex and race

AGE	MALE						FEMALE					
	White			Negro			White			Negro		
	T	N	%	T	N	%	T	N	%	T	N	%
1-9	1	0	0	0	0	0	0	0	0	0	0	0
10-19	8	0	0	23	1	4.4	3	0	0	16	0	0
20-29	72	4	5.6	214	3	1.4	20	0	0	107	3	2.8
30-39	200	14	7.0	317	6	1.9	43	2	4.7	120	0	0
40-49	427	29	6.8	306	13	4.3	40	0	0	102	1	1.0
50-59	461	24	5.2	232	5	2.2	56	0	0	77	1	1.3
60-69	454	36	7.9	148	5	3.4	73	3	4.1	55	0	0
70-79	253	14	5.5	69	4	5.8	71	1	1.4	42	0	0
80-89	67	2	3.0	21	2	9.5	34	1	2.9	24	1	4.2
90-99	3	1	33.0	2	0	0	4	1	25.0	4	0	0
100-	0	0	0	0	0	0	0	0	0	2	0	0
Unknown	6	1	16.6	7	0	0	4	0	0	12	0	0
Totals	1952	125	6.4	1339	39	2.8	348	8	2.3	561	6	1.1
49 and under	708	47	6.6	860	23	2.7	106	2	1.9	345	4	1.2
50 and over	1238	77	6.2	472	16	3.4	238	6	2.5	204	2	1.0

T indicates the number of lumbar columns examined; N, the number of these columns showing one or more neural arch separations; %, the percentage of separations.

178. Since in 5 of these, there was more than one separate neural arch, the total number of separate neural arches was 183. In determining the number of times a particular bone variation occurred within the 178 lumbar columns showing

TABLE 2

Incidence of certain vertebral characters coincident with separate neural arch

CHARACTER STUDIED	N	C	%
1. Atypical number of presacral vertebrae	158	12	7.5
Twenty-three		4	2.5
Twenty-five		8	5.0
2. Facets for cervical ribs on C ₇	174	4	2.3
3. Spina bifida			
Atlas	158	3	1.9
C ₆ , C ₇ , T ₁	158	1	0.6
Lumbar 5	153	9	5.9
with unilateral separation		6	3.9
with bilateral separation		3	2.0
Lumbar 6 (with bilat. separation)	2	2	
Sacrum	172		
complete upper segments only		23	13.4
open dorsal bony wall of canal		7	4.1
4. Unilateral isthmal separation and separation through opposite lamina	153	3	2.0
5. Transverse processes of L ₅ articulating with ilium and/or sacrum	171	3	1.8
6. Arthritic manifestations			
Lumbar articulator processes	176	57	32.4
mild		27	15.3
moderate		23	13.1
severe		7	4.0
Central atlantoepistrophe joint	170	91	53.5
mild		53	31.2
moderate		30	17.6
severe		8	4.7
7. Accessory sacroiliac articulations	172	27	15.7
8. Incomplete fusion of S ₁ and S ₂	172	7	4.1

N indicates observations which were made for each particular character; C, number of times the character occurred; %, percentage incidence.

separate neural arches, it must be noted that on occasion that portion of a column in which the variation or character might occur was either defective or missing, and for this reason, the number of observations will be found to vary from 153 to 176.

Table 3 reveals the specific locations and types of the neural arch separations found, and indicates, as is already known, that the 5th lumbar vertebra was involved most frequently. Each preceding segment was involved less frequently until the first lumbar segment was reached in which no separations were found. Separations occurred bilaterally in approximately five-sixths of the columns involved whereas the re-

TABLE 3

Incidence of separate neural arch according to lumbar vertebra and side

VERTEBRA	RIGHT SIDE ONLY		LEFT SIDE ONLY		BILATERAL		TOTAL	
	No.	%	No.	%	No.	%	No.	%
L ₁	0	0	0	0	0	0	0	0
L ₂	2	1.1	1	0.5	0	0	3	1.6
L ₃	1	0.5	0	0	4	2.2	5	2.7
L ₄	0	0	1	0.5	16	8.8	17	9.3
L ₅	19	10.4	8	4.4	129	70.5	156	85.3
L ₆	0	0	0	0	2	1.1	2	1.1
Total	22	12.0	10	5.5	151	82.5	183	100

maining one-sixth of the separations were unilateral and distributed twice as frequently on the right as on the left side.

DISCUSSION

The conclusion which is here drawn from the comparison of the incidence of neural arch separations and age does not differ from that drawn previously by Willis ('31) and Friberg ('39). However, as stated before, the present results were obtained by a different method than that used by these workers. It should be remembered that failure to demonstrate changing incidence with age extends only through the age period of approximately 20 to 80 years. In the present study

there was not sufficient material to justify conclusions regarding the relation of age and incidence outside of this range.

The incidences of neural arch separation with reference to race have been pointed out frequently before, and are repeated in table 4 with the addition of most of the large series

TABLE 4
Incidence of lumbar neural arch separation according to race

RACE	SOURCE	NO. OF OBSERV.	NO. OF EFFECTS	PER CENT DEFECTS
<i>Negroes</i>				
Bantu Natives	Shore ('28-'29)	56	5	8.9 ³
American	Present study	1,900	45	2.4
	Total	1,956	50	2.5
<i>Whites</i>				
Central European	Hayek ('28)	200	6	3.0
	Schwegel (1859)	100	4	4.0
American Whites	Present study	2,300	133	5.8
	Total	2,600	143	5.5
<i>Mongols</i>				
So. American Indians	Ten Kate ¹	102	5	4.9
No. American				
Aborigines	Congdon ('32)	200	10	5.0
Japanese	Hasebe ('12-'13)	125	7	5.6
	Taguchi ²	97	10	10.3
	Adachi ²	65	7	10.8
Eskimos	Stewart	350	96	27.4
	Total	939	135	14.4

¹ Quoted from Stewart ('31-'32).

² Quoted from Hasebe ('12-'13).

³ The percentage, as given by Shore was 9.1%. However, on the basis of his given figures it appears to be 8.9%.

found in the literature (Congdon, '32; Hasebe, '12-'13; Hayek, '28; Schwegel, 1859; Shore, '28-'29; Stewart, '31-'32). The series of Willis ('31) was not included because it was derived from a portion of the skeletons used in this study and, therefore, would represent repetition in part. It is apparent from table 4 that the Negro race presents neural

arch separations considerably less frequently than the White race, and the White race less frequently than the Mongols. The highest incidence reported is that found by Stewart in the Eskimo (Stewart, '31-'32), but even if this special group were eliminated from the Mongols, the incidence in Mongols is still higher than in either of the other races considered.

Review of the figures from the present series in relation to incidence and sex indicated that the condition was approximately three times as frequent in males as in females. In the very early literature when neural arch separations were detected only because of associated spondylolisthesis they were described exclusively in females because of the difficulty which resulted in labor due to the obstruction of the pelvic outlet by the displaced vertebral body (Kilian, 1854; Neugebauer, 1888). In these cases all too frequently post-mortem study established unequivocally the nature of the obstructing mass whose identity had been surmised only imperfectly from pelvic examination. The current tendency among clinicians is to regard the condition as more frequent among males (Meyerding, '41) and the present figures support this conclusion.

Coincident bone variations. Since Gregg's discovery in 1941 that the occurrence of rubella in a pregnant woman might produce cataract and congenital heart disease in the infant resulting from the pregnancy, numerous papers have appeared which have confirmed and extended his observations (Wesselhoeft, '47). Thus, an understandable basis for the frequent occurrence of multiple congenital variations is rapidly being accumulated (Gruenwald, '47; Warkany and Nelson, '41; Wesselhoeft, '47; Wiener, '47). In the light of this known association of congenital variations in the same individual it seemed appropriate to search the vertebral columns which presented a separate neural arch for the presence of certain other bone variations, and when possible to compare the incidence of these coincident variations with the incidence already established in unselected groups of skeletons. If the separate neural arch could be shown to occur in those col-

umns which presented several congenital variations one might be inclined to think of it too as a congenital variation, whereas the failure to observe such an association might bear some weight against this concept. It must be emphasized that such reasoning cannot be applied to a congenital variation which of itself might predispose toward instability or weakness of the involved vertebra or on the other hand to those variations which yield greater stability to the region in which they occur.

Because they are derived from a heterogeneous group, the figures concerning the present investigation of the number of presacral segments (table 2) cannot be compared statistically with the most accurate counts previously recorded. However, it appears that there is no significant difference between the incidence in the present series of 2.5% with 23, 92.5% with 24, and 5.0% with 25 presacral vertebrae and those obtained by Lanier ('39) on 100 normal White male and 100 normal Negro male skeletons. In the Whites he found 3.0% with 23, 95% with 24, and 2.0% with 25 presacral vertebrae. The respective figures for the Negro males were 5.0%, 88.0% and 7.0%. The present figures correspond closely too with those in Bardeen's summary of the literature ('04) in which there were 4.3% with 23 presacrals and 4.4% with 25.

Articular facets for cervical ribs were present on the 7th cervical vertebra in 4 cases or 2.3% of 174 columns in good enough condition to so determine. This compares favorably with the 2.0% incidence found by Lanier ('39), and Todd's finding ('12) of 1.16% incidence. Such articular facets were not observed on cervical vertebra other than the 7th but a special search for them was not made in view of their rarity (Webb and Brown, '21).

Four columns presented spina bifida of the cervical vertebrae. In three of these the atlas was involved, thus constituting 1.9% of the 158 columns with all 7 cervical segments or almost the same percentage as was found by Lanier ('39) for both White and Negro males (2%). In one case spina

bifida was present in the 6th and 7th cervical and the first thoracic vertebrae. The 5th lumbar was involved with spina bifida plus neural arch separation 9 times or in 5.9% of the 153 satisfactory specimens (fig. 1). (The total number of satisfactory specimens for this examination is somewhat lower than most in this series because of the frequent loss of the separated segment comprising the inferior articular processes, laminae and spinous process.) This incidence is about 10 times the expected rate as determined by Lanier ('39) for the 5th lumbar vertebra on the basis of examination of 100 White and 100 Negro male skeletons, and $4\frac{1}{2}$ times the frequency as determined by Willis ('23-'24) for the last lumbar vertebra in 748 lumbosacral columns unselected as to race and sex. The lumbar vertebrae other than the 5th presented no spina bifida except for the two cases of bilateral separation through the 6th lumbar, in both of which the separated fragment was bisected by a central separation, i.e., spina bifida.

In 5 specimens, bilateral separation through the isthmus was associated with spina bifida of the separated arch, whereas 6 cases of unilateral isthmal separation occurred in conjunction with spina bifida of the involved arch and three more cases of unilateral isthmal separations occurred with a defect through the opposite lamina. Thus, 5.1% of the total involved columns presented a unilateral isthmal separation combined with a break through the neural arch in another point, or 28.1% of the unilateral isthmal defects were associated with another separation through the arch. Of the 151 bilateral separations the separated segment was missing in 25 instances and, thus, only 126 remained satisfactory for study as to incidence of spina bifida of the separated fragment. Five or 4% of these separated segments demonstrated spina bifida.

Enlarged transverse processes of the 5th lumbar vertebra were found to articulate with the sacrum or ilium or both in three of 171 cases satisfactory for examination or in 1.8%. Similar articulations were reported by Moore ('24) to occur

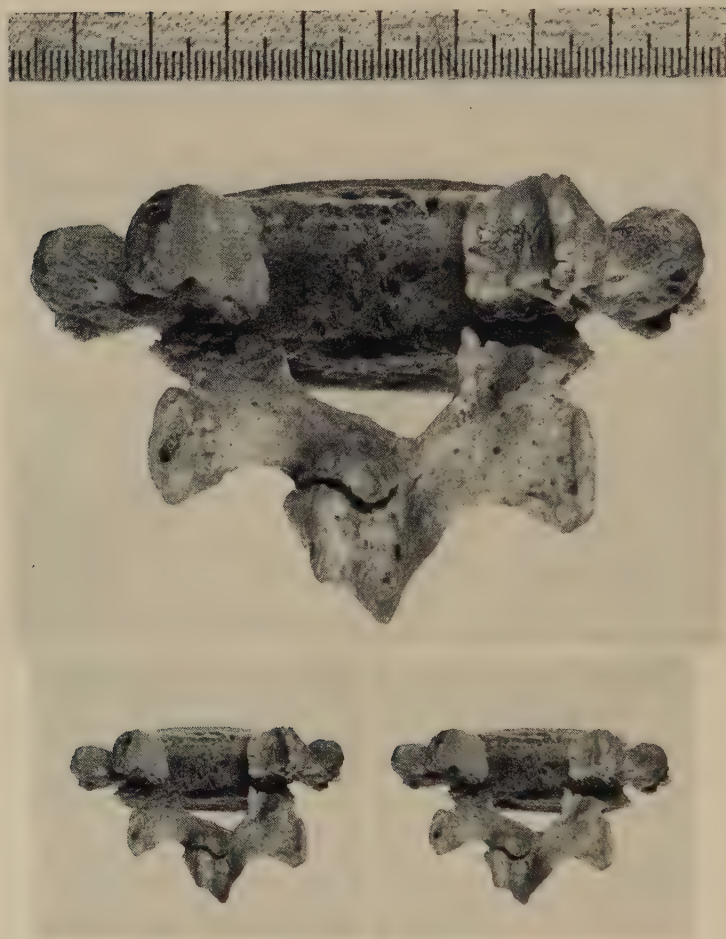


Fig. 1 The upper figure presents a 5th lumbar vertebra in which a bilateral isthmal separation is combined with spina bifida of the separated fragment. The lower two figures constitute two stereographic pairs. The most satisfactory stereographic image is obtained by holding a blotter or stiff paper as a partition upright between the two figures and observing them so that it is possible to see only the right image with the right eye and the left image with the left eye. When viewed thus at approximately 10 inches, two images are seen at first but if one gazes in the distance through the images they move together and fuse into one which is then seen in three dimensions. At first attempt the fused images are apt to fly apart when accommodation for detailed study it attempted because of the convergence which normally accompanies accommodation but subsequent attempts become progressively more successful. (Upper figure natural size, scale in centimeters.)

in 6% of unselected skeletons and by Lanier ('39) to occur in 6.0% of White males and 8.0% of Negro males, or approximately 3 to 4 times as frequently.

Observations were made on 172 satisfactory sacra. Of these, spina bifida of the first sacral segment was found in 30 or 17.4%. This is essentially the same as that reported by Adolphi ('11) since he recorded 48 cases of spina bifida involving the first sacral segment with or without other segments in the same sacrum out of 292 sacra or an incidence of 16.4%. The present observations revealed incomplete spina bifida of the first sacral segment in 10 additional cases or 5.8%. Of the 30 sacra in this series with spina bifida of S₁ the posterior wall of the sacral canal was open throughout in 7 cases or 4.1% of the total sacra. This incidence of the open type of sacrum is approximately two times as frequent as was found previously by Trotter ('47) in unselected groups of bones, since she reported an incidence of 2.0% in males and 0.3% in females. Accessory sacroiliac articulations were observed in 15.7% as compared to the previously established incidence of 50.5% in White and 20.7% in Negro skeletons selected at random by Trotter ('37). Incomplete fusion of the first with the second sacral segment was present in 7 or 4.1% of these sacra. A rudimentary superior articular process of the first sacral segment was demonstrated in 4 or 2.3% of the 172 sacra in this group.

Arthritis of articular processes of the lumbar vertebrae. If chronic trauma plays any significant role in the etiology of the separate neural arch, it was felt that perhaps in those columns in which the separations occurred, there might be found greater degrees of hypertrophic or traumatic arthritic changes, at least at the level in association with the defects. Examination carried out from this standpoint revealed arthritic involvement of the lumbar articular processes which was considered to be mild in 27 or 15.3%, moderate in 23 or 13.1% and severe in 7 or 4.0% of the total 176 satisfactory specimens. Thus, 32.4% presented some arthritic involvement of the lumbar facets and 67.6% were normal. Break-

down of these by age revealed that only one case of arthritis and that one of mild degree occurred before the age of 40 and that subsequent to this there was an increase of the frequency and severity of the arthritic manifestations with increase in age. Lipping of the central atlantoepistrophic joint was observed to be mild in 31.2%, moderate in 17.6% and severe in 4.7%. Thus, 53.5% of the 170 specimens satisfactory for this examination showed some degree of involvement. Unfortunately, no figures with which these can be compared have been found, since it is believed that the control group would have to be chosen from the same race, sex and age as well as social level in order to be a valid comparison. However, these results for the lumbar vertebrae are very similar to those reported by Putti and Logroscino ('37) in which the incidence and severity of arthritic change of the articular processes was shown to increase progressively with age. Shore's observations ('34-'35) of the same process in vertebral columns was made on 125 skeletons whose age, race and sex were unknown, and, therefore, seems unsuitable for determining comparative frequency between the present series and the expected frequency in unselected bones. In the same group of skeletons Shore found that 32.2% presented arthritic changes at the central atlantoepistrophic joint, but again this must be compared only with reservation to the figures derived from this series because the age of the two groups may well be significantly different. It is believed that no great etiologic significance can be assigned to a condition which is known to be absent in over two-thirds of cases with neural arch separations. Furthermore, involvement of the cervical joint studied revealed arthritic manifestations to be almost twice as frequent in that area as in the region of the column presenting a neural arch separation.

Incidence of separate neural arch according to segment level. The present observations confirm the clinical finding that the majority of isthmal separations occur in the 4th and 5th lumbar vertebrae. The specific figure for separations in these two segments was 94.6% of the total number of de-

fects. The first lumbar vertebra presented no separations, there were 3 in the 2nd lumbar vertebra, 5 in the 3rd, 17 in the 4th, 156 in the 5th, and 2 in the 6th lumbar vertebra, within the total of 183 isthmal defects. This tendency for the incidence of the defect to increase as the lumbosacral joint is approached is confirmed by the results of most other workers (Stewart, '31-'32). It should be emphasized that the generally reported low percentage incidence in the 6th lumbar vertebra including the present series on the surface is misleading. It should be remembered that only 3.2% of skeletons can be expected to present 6 lumbar vertebrae (Bardeen, '04), and, therefore, the incidence of neural arch separations which is calculated from the total number of columns examined includes very many columns in which there was no 6th lumbar vertebra.

Hasebe ('12-'13) recorded two neural arch separations in the cervical region, and Stewart ('32-'33) reported one. Friberg ('39) has reported roentgenographic demonstration of separations in the thoracic portion of the vertebral column and Congdon ('32) reported one isthmal separation in the first sacral segment. In the present study none were found in any portion of the column other than the lumbar. Failure to observe separations at other levels in the present series may have been the result of the method of investigation since the whole vertebral column was not examined except in those cases which presented neural arch separations in the lumbar vertebrae. It can be stated that in the 178 complete columns examined, no such defects were found and that Lanier ('39), in his study of 200 presacral columns, mentioned no separations at such other levels. There is sufficient evidence to conclude that such separations are infrequent in sections of the column other than the lumbar.

Multiple segment involvement. Neural arch separations in two vertebrae of the same column occurred in 5 cases or 2.8% of the 178 columns presenting separations and 0.12% of the 4,200 lumbar columns examined. In these multiple lesions, both separations were bilateral in three cases and

a bilateral separation was combined with a unilateral separation in two cases. Although most of the multiple involvements were found in adjacent vertebrae, in one case they were widely separated. Thus, bilateral lesions were found to involve both lumbar 4 and 5 in the same column in 2 specimens and lumbar 3 and 4 in the 3rd. In another specimen, there was a bilateral defect in lumbar 5 and a unilateral defect in lumbar 4, whereas in the 5th case a bilateral separation in lumbar 5 was combined with a unilateral separation in lumbar 2. No special features could be distinguished which set these columns apart from those with only one separation except that they all occurred in males. This may not be significant since there were approximately $3\frac{1}{2}$ times as many males as females in the material studied, and the males had more than 13 times as many separations as the females.

Two vertebrae was the greatest number involved in any one column. The greatest number of involved vertebrae found in the literature was cited by Friberg ('39) whose case was that of an 11 month old girl with bilateral club foot, subluxation of both knee and elbow joints, dislocation of both hips and funnel chest, as well as isthmal separations of all the lumbar and the two lower thoracic vertebrae and spina bifida of lumbar 4 and 5. On the other hand, by far the most frequent occurrence of multiple involvements was found by Stewart ('31-'32) in Eskimo skeletons.

Variation in pattern of defects. There were numerous variations in the pattern of involvement, not only as far as morphology of the actual separation was concerned but also in the degree of involvement. It was noted that the extent of the lesion varied from a thin unilateral loss of continuity which was no more than a line through the bone (fig. 2) to a wide bilateral defect in which nothing that could be considered isthmus was present (fig. 3). In some cases, the defect extended through what appeared to have been a normal isthmus, but in others the involved isthmus was very thin and appeared to have been weak. At times the separated fragments could be fitted together like parts of a jig saw

puzzle in which the projections from one side corresponded to the depressions of the opposing surface, but in others large spaces were left between protruding serrated edges which did not interdigitate with each other. In all cases the adjacent ends of the fragments were smoothed over so that no chance of confusion with post-mortem fracture existed.



Fig. 2 Stereographic pairs chosen to demonstrate a narrow unilateral isthmal separation. Note how little deformity is present, and how narrow is the line of separation (approximately one-third size).

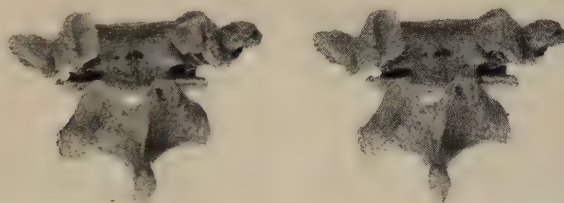


Fig. 3 These figures are stereographic pairs of a 5th lumbar vertebra chosen to demonstrate a wide bilateral separation in which almost none of the isthmus remains. Note the thin, sharp superior margin of the separated fragment (approximately one-third size).

Thirty-two or approximately one-sixth of the 183 separations were unilateral. Thus, 0.76% of the 4,200 skeletons presented unilateral isthmal separations. These were twice as frequent on the right as on the left. Unilateral separations constituted 6.7% of the total separations found in Negroes and 21.0% of those found in Whites. Regarding sex incidence, 7.2% of the separations in females were unilateral

as compared to 18.3% in the males. A striking observation with regard to the unilateral separations was the twisted appearance of those vertebrae in which they were found. In almost all cases encountered, the arch was asymmetrical apparently due to the increased length of the defective isthmus. Hence those with wide separations were more asymmetrical than those with narrow separations.

Bilateral neural arch separations were found in 3.6% of the total number of skeletons observed, and constituted five-sixths of the total separations. Although no bilateral lesions were found in the first two lumbar segments, the incidence mounted steadily from lumbar 3 through 5. The more uniform and consecutive arrangement of bilateral lesions as compared to the unilateral ones (table 3) is due possibly to the smaller number in the latter group. Of the total separations found in Negroes, 93.3% were bilateral as were 79.0% of those in Whites. Ninety-two and eight-tenths per cent of the separations in females and 81.6% of those in males were bilateral. No significant difference could be demonstrated between the ages of those with bilateral separations and those with unilateral separations.

SUMMARY

1. A study of the separate neural arch and coincident bone variations as found in human skeletons is reported.

2. Of the 4,200 skeletons 178 had neural arch separations establishing an over-all incidence of 4.2%. On the basis of race and sex, the incidence is further delineated: White males 6.4%, Negro males 2.8%, White females 2.3% and Negro females 1.1%.

3. The incidence of the separate neural arch is shown to remain almost constant regardless of age in such age periods (adult life) as covered by this skeletal material.

4. In this series of columns with isthmal separations the coincident bone variations studied whose incidence was significantly different from determination in the normal were as follows: spina bifida of the 5th lumbar vertebra was ap-

proximately $4\frac{1}{2}$ times as frequent as is normally expected; the open sacrum was approximately twice as frequent; articulation of the transverse process of the 5th lumbar vertebra was approximately one-third as frequent; accessory sacroiliac articulations were approximately one-half as frequent as the normal expectancy.

5. Those coincident bone variations studied in this series whose incidence was essentially the same as that found in unselected bones were as follows: atypical number of pre-sacral vertebrae, cervical ribs, spina bifida of vertebrae other than the 5th lumbar, spina bifida of the upper sacral segments.

6. Arthritic involvement of the lumbar vertebral articular processes increased in frequency and severity with increasing age, but was absent to gross inspection in over two-thirds of those columns showing isthmal separations.

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ANOMALOUS CENTERS OF OSSIFICATION FOR INFERIOR ARTICULAR PROCESSES OF THE LUMBAR VERTEBRAE

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TWO FIGURES 

During the course of the dissections of 53 stillborn human fetuses and examining 20 human embryonic and fetal specimens cleared and stained by the Spalteholz method¹ in a study of ossification of the neural arch of the lumbar vertebrae an instance of accessory anomalous ossification center in the region of the right inferior articular process of the second and third lumbar vertebrae was discovered. This is thought unusual enough to merit a brief report.

In the older works of embryology, at least since 1864, one frequently encounters references to the normal ossification pattern as including two ossification centers for each half of the neural arch (Bardeen, '10; LeDouble, '12; Poirier and Charpey, '11). It is believed that many of these references have been derived from the work of Rambaud and Renault (1864), in which such findings were reported. The latter authors stated in their treatise that their results differed from the previous observations of Fallopius, Coiter, Riolan, Spigel, Kerckringe, Albinus and Beclard and their results have continued to differ from the results of most later studies. The centers of ossification described by Rambaud and Renault and by the others who agreed with them, included for each side one for the superior articular process and pedicle, and a second for the inferior articular process, lamina and one-half of

¹ These specimens were collected and prepared by Dr. J. K. Woo.

the spinous process. Thus, including one for the body, there were said to be 5 primary centers in each vertebra.

According to present-day textbook descriptions there are only three primary centers of ossification in each vertebra. One appears in the body, and one in each side of the neural arch at about the 7th or 8th week of fetal life. Ossification extends anteriorly and posteriorly from those centers which appear in the neural arch and the right and left bones fuse with each other posteriorly at approximately one year of age. The bony pedicles, however, do not fuse with the body until between the 3rd and 6th year, and thus, until that time a neurocentral synchondrosis exists bilaterally in each vertebra. Secondary centers for epiphyses at the ends of the transverse and spinous processes, on the superior and inferior surfaces of the bodies, and for such specialized parts as the mammillary processes of the lumbar vertebrae, appear at approximately the 16th year of life and these epiphyses fuse to the adjacent parts of the vertebra at about the 25th year (Arey, '46; Goss, '48; Grant, '48; Patten, '46; Spalteholz; Terry, '42).

Whereas frequent variation in the ossification pattern might be expected so far as the neural arch is concerned, a review of the literature reveals that in studies of 509 fetal spines including the present series with those collected from papers in which the number of observations was recorded (Batts, '39; Chandler, '31;² Friberg, '39; Hitchcock, '40; Mall, '06; Noback, '43, '44; Sensenig, '49), and in several studies where the total number of observations were not recorded (Bardeen, '05; LeDouble, '12; Hayek, '28; Willis, '31) only one case of an accessory center for a part of the neural arch has been reported. This was described by Batts ('39) whose studies embraced a series of 200 fetal spines. The accessory center existed in the third lumbar vertebra on the right side and was in the region of the inferior articular process.

It should be noted that the secondary centers reported by Willis ('31) (and demonstrated by photographs) were located

² Number of examinations quoted from Batts ('39).

in the last lumbar vertebra in a position comparable to the lateral masses of the sacral segments. This location is quite far removed from that of the inferior articular process where the center reported by Batts and the two centers reported here were found.

The anatomical variations under consideration was found in a Negro male stillborn at approximately 7½ months of gestation. The lumbar vertebrae were exposed through a posterior midline incision and examined after elevation of the periosteum and perichondrium from the neural arches. The second and third lumbar vertebrae on the right side each presented a small, round, accessory bony center for the inferior articular process, adjacent to, but distinctly separated by cartilage from, the center for the superior articular process, pedicle, and lamina (figs. 1 A, B, C, and D). It is evident from the continuity of the developing lamina with the superior articular process (fig. 1 A), and its proximity medially with the opposite lamina, that if fusion of the anomalous center with the rest of the arch did fail to occur, the arch would still be continuous apart from it. The result would be only an articular process separated from the rest of the neural arch. This report and the previous one in the literature in no sense, therefore, confirm the work of Rambaud and Renault.

Our observations bring the recorded total cases of fetal and stillborn spines examined in recent years to 509 with 2 or 0.4% presenting such anomalous accessory centers. This percentage is possibly too high because of the examinations done without recording the total cases observed or possibly too low because of reported series in the literature which were not found. It is of interest that the three accessory centers which have been found in processes of the neural arch occurred in the upper lumbar vertebrae (two and three) rather than within the neural arch of the 5th lumbar vertebra.

Such accessory centers as reported here could play no role in the genesis of separate neural arch (spondylolysis, spon-

dyloschisis) because the bony bridge from one superior articular process to the other would be complete without them. Nor do they seem to bear any relation to the small accessory ossicles described by Hipps ('39) as occasionally seen separated from the inferior extremity of the inferior articular

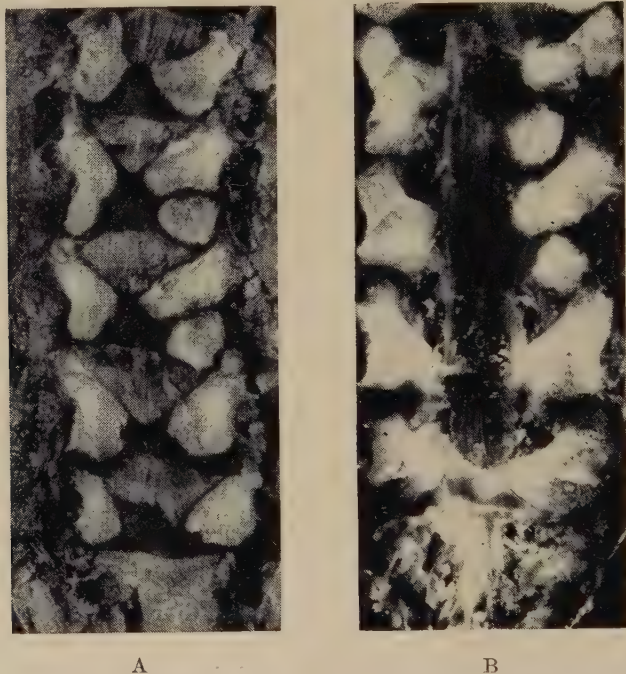
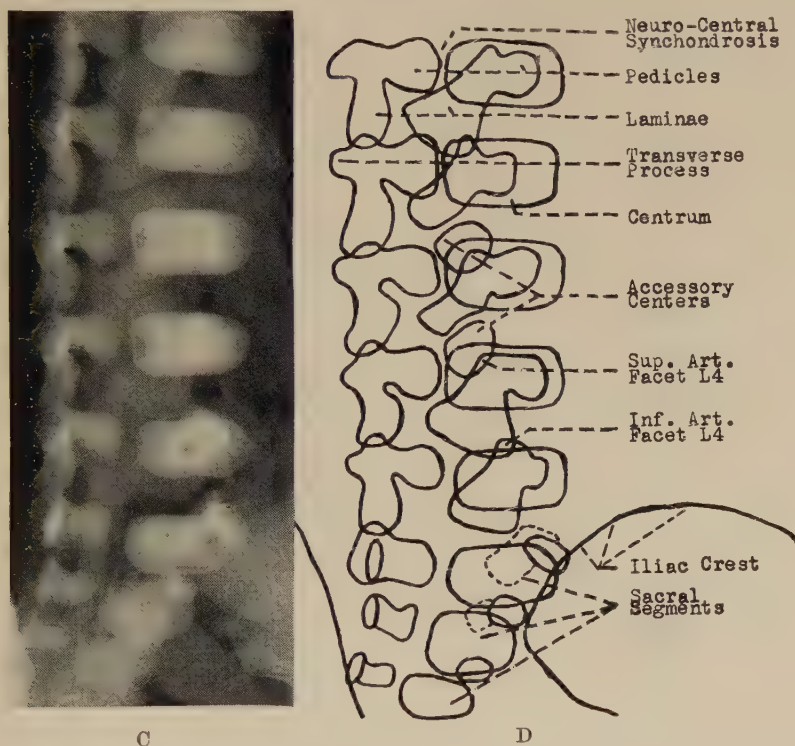


Fig. 1 A Posterior view of a dissection of a $7\frac{1}{2}$ month old fetus showing the lumbar spine with anomalous accessory centers for the right inferior articular process of the second and third lumbar vertebrae. Cartilage, connecting the laminae across the midline appears dark and homogeneous, ligament striated and bone quite light. Note the development of the right lamina and superior articular process of lumbar 2 and 3 as one bone mass; also the proximity of the lamina to its opposite fellow. (Magnification of all reproductions in figure 1 about three times. 1 A and B retouched for clarity.)

B Posterior view of the same fetal spine after clearing with potassium hydroxide and suspension in glycerine. The supporting cartilage is no longer visible, and the bone is seen clearly outlined against the investing membranes of the spinal cord below. Slight displacement of the upper accessory center occurred in the clearing process. The first lumbar laminae are barely visible cut through at the extreme top of the figure.

process of the lumbar vertebrae of the adult, for the ossification centers here described are much larger in proportion to the rest of the neural arch than are the ossicles of the adult, and they appear to represent the whole inferior articular process instead of an accessory portion. The unusual condition of rudimentary or absent inferior articular process (Rowe, '50) is very probably due to failure of such an accessory center as described here to develop fully or to become attached to the rest of the neural arch.



C Right postero-oblique roentgenogram of the same fetal spine demonstrating the anomalous accessory centers and their relation to the rest of the neural arch.

D Line tracing of roentgenogram presented in 1C. Only the bone has been outlined to make for maximum clarity. Ossification centers for the arch normally begin between the superior and inferior articular processes and spread postero-medially through the lamina and anteriorly through the pedicle.

SUMMARY

1. A case of right, unilateral accessory ossification centers for the inferior articular process of the second and third lumbar vertebrae is presented.

2. A survey of 509 fetuses in the literature whose lumbar vertebrae were studied for pattern of ossification revealed one similar instance, except in that case only one accessory center was found.

3. The possible relation of this finding to certain conditions seen in the adult is discussed.

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AMERICAN ASSOCIATION OF ANATOMISTS
Sixty-fourth Annual Session

Wayne University College of Medicine
Detroit, Michigan

March 21, 22, 23, 1951

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ABSTRACTS

Abstracts of papers presented at the 64th meeting of the American Association of Anatomists at Wayne University College of Medicine, Detroit, Michigan, March 21, 22, and 23, 1951.

The abstracts are listed in alphabetical sequence by senior author in 4 lists as follows: papers presented from platform, papers read by title, demonstrations, and motion picture demonstrations.

The numbers at the beginning of the abstracts refer to the order of presentation at the meetings, as printed in the program.

PAPERS FROM PLATFORM

17. *Variations in the portal system of veins.* William F. ALEXANDER and Harry K. PURCELL, Departments of Anatomy and Surgery, Saint Louis University, School of Medicine.

The major tributaries of the portal system have been dissected, mapped and measured. Four predominant patterns were found in 132 cadavers. These pat-

terms are based upon the variability of the entrance of the inferior mesenteric and the left gastric (coronary) veins into this system. In type one, the most frequent, the inferior mesenteric vein drained into the superior mesenteric vein. This was found in 53% of the cadavers. In this same type the coronary vein was a tributary of the portal vein, found in 65%. Forming type two, the inferior mesenteric vein joined the splenic vein in 31% of the bodies, whereas the coronary vein joined the splenic in 22%. Twelve per cent of the dissections made up type three. In this type the inferior mesenteric vein bifurcated. One branch entered the splenic vein and the other the superior mesenteric. Type four was found in 3% of the bodies. The inferior mesenteric vein in this type joined the point of junction of the superior mesenteric and the splenic veins to drain into the portal vein. Measurements of lengths and diameters of these veins were made. There was no significant difference between the fresh and the embalmed cadaver.

89. *New information on lymphocytes in normal epidermis.* Warren ANDREW, Department of Anatomy, the George Washington University.

Further studies have been made on the presence of lymphocytes in the normal epidermis, which previously had been affirmed by the author for rat and man. Observations on the numbers, distribution and appearance of these cells at different ages in rats, including immaturity, youth, middle-age and senility, have been made. The results of quantitative and qualitative investigation indicate the constancy and general similarity of the phenomenon at all ages in the rat. The histological findings support the view earlier proposed by the author that a constant migration and differentiation of lymphocytes into clear cells and on to epithelial cells does occur. No evidence of degeneration of lymphocytes in the epidermis or of their movement to the more superficial layers has been found. Data are given also on counts of mitotic figures. The epidermo-dermal boundary also is described as seen by specific stains for the reticular connective tissue and the absence of any barrier to cell migration is confined. Observations on lymphocytes in normal epidermis have been extended to several other species of mammals, demonstrating the widespread occurrence of the phenomenon and the similarity in details of the cellular relationships.

66. *The development of the otic capsule in the region of the vestibular aqueduct.* Barry J. ANSON, Department of Anatomy, Northwestern University Medical School, and Theodore H. BAST, Department of Anatomy, University of Wisconsin.

As already established in an earlier phase of the investigation, the endolymphatic (otic) sac, unlike the remainder of the membranous labyrinthic system, continues to enlarge until the time of birth; and during growth, change in position occurs toward a transverse plane.

As a consequence, the region of the aqueduct undergoes morphogenesis after the otic capsule generally has attained adult size and histologic stability. The

steps in the development may be traced in five stages selected from numerous series studied.

In the 150-mm fetus, bone has appeared on the lateral wall of the vestibular orifice, while the medial wall is still cartilaginous.

Periosteal bone in the 183-mm fetus has spread from the dural aspect of the capsule to the aspect which faces the endolymphatic sac. Therefrom, in the fossa of sac, the wall is constituted of combined intrachondral and endochondral bone.

In the 215-mm fetus, while periosteal bone has undergone considerable increase in thickness, the middle layer, within the fossa, has experienced erosive deepening.

In the 270-mm stage, periosteal bone forms a secondary layer; it partially fills the one capacious space of the orifice.

Adult configuration and size are attained in the infant; periosteal bone then lines the entire cranial orifice of the vestibular aqueduct.

73. *On the change in position of growing follicles in the mammalian ovary.*

L. B. AREY and E. J. CUMMINS, Department of Anatomy, Northwestern University Medical School.

This study treats of the degree of correlation existing between the depth of position of growing follicles in the mammalian ovary and their sizes, and with the occurrence and orientation of localized bulges of the theca interna, known as cones or wedges, with respect to such growing follicles. The previous factual conclusions of some workers in these matters and their interpretation of the cone as a specialized instrument by which the follicle rises from a deeper to a more superficial position are either not supported or are modified by the restudy to be described. The present conclusions have been reached both on the basis of direct observation and by a statistical treatment of a greater amount of material than has hitherto been analyzed by others. This material includes the rat, guinea pig, cat, dog and monkey; the present report is based specifically on the ovaries of the macaque monkey.

9. *Thyroid gland destruction with astatine.* C. W. ASLING, J. G. HAMILTON, K. G. SCOTT, P. C. WALLACE, W. M. GARRISON, G. P. THILO and D. C. MORRISON, Divisions of Anatomy, Experimental Medicine, Radiology, and Medical Physics, Crocker Laboratory, and Institute of Experimental Biology, University of California.

Astatine (At^{210} , "eka-iodine") was prepared in the cyclotron and injected into adult rats. Uptake of the radio-element by the thyroid gland was demonstrated by subsequent radio-assay of the tissue. Radioautographs showed it in follicular epithelium and colloid. Varying dosage levels and post-injection intervals were studied, and thyroid damage assessed histologically and by radioautographic demonstration of impaired radioiodine uptake. Occasional damage to the thyroid occurred with 10 microcurie doses. Nearly complete follicular

destruction and fibrous replacement was found a month after injection at levels near 50 microcuries. Even at the highest dosage levels no change in parathyroid structure was demonstrated. The alpha-emission of astatine may account for its effectiveness at lower dosages than beta-emitting iodine. Varying the valance states did not affect the uptake by the thyroid:

Marked sub-total destruction of monkey thyroid was also demonstrated.

158. *The median hypothalamic nuclei in some mammals.*¹ J. AUER, Department of Anatomy, University of Ottawa.

After studying the postnatal development of the hypothalamic nuclei in the golden hamster (Auer, '50), it was felt desirable to investigate the adult pattern, particularly of the median hypothalamic nuclei. Also a comparative study of these nuclei has been carried out on serial sections of brains from the adult hamster, rat, guinea pig, rabbit, cat and monkey. These sections have been stained with the Nissl, Bodian and Heidenhain methods.

The observations have revealed that the midline nuclei are most conspicuous in the hamster, though they are also present in other rodents. In the cat and the monkey the differentiation of the bilateral nuclei seems to have prevented the appearance of some midline nuclei. However, the median preoptic nucleus, the median infundibular nucleus and the median arcuate nucleus are seen in all species examined (see also Krieg, '48). The median suprachiasmatic nucleus is very variable in size, while the median mammillary nuclei seem to be differentiated only in rodents, particularly in the hamster.

The individual variation of the nuclear pattern in the hypothalamus is obvious. This was already found in postnatal stages of the hamster and was also stressed by Gurdjian ('50). The observations have served as a suggestion for experiments inflicting lesions in the midline in order to investigate the connections of this region. These experiments are now under way.

¹ Supported by a grant in aid of research of the National Research Council, Canada.

186. *The effects of total body roentgen ray irradiation on the nucleic acids of rats.*¹ Daniel L. AZARNOFF, S. W. LESHER and Paul G. ROOFE, Department of Anatomy, University of Kansas School of Medicine.

Adult male Sprague-Dawley rats were divided into two groups. Group A received 412.5 r and group B, 600 r x-ray irradiation. Normal animals and also control animals pair-fed with irradiated animals were studied. Five days post irradiation all animals were sacrificed. Sections of testis, liver, thyroid, kidney, adrenal and pancreas fixed in Carnoy (3-1) were analyzed for nucleic acids by toluidin blue, Brachet ribonuclease technique and Feulgen reaction.

Irradiation changes in both nuclei and cytoplasm with their respective nucleic acids, DNA and RNA, are found. In nuclei, chromosomes become pyknotic and nuclear sap in these nuclei gives basophilic reaction. In more heavily damaged organs this is followed by death of the cell and subsequent cytolysis. Irradiation damage, however, is neither equal nor the same in all organs. Overall damage appears to be greatest in the testis where not only are the nuclei pyknotic,

but the cells themselves are apparently fragmented and basophilic debris fills the lumen of the seminiferous tubules. Sever damage, but to lesser degree than in testis, is found in liver, thyroid and kidney. In pancreas, injury is scarcely evident. In each organ studied, damage is not overall, but attacks specific regions, e.g. atrophy of the glomeruli and necrosis of nephron units in the kidney.

Tissue of pair-fed control animals presents a stimilar pattern, but damage is to a much lesser degree.

¹ This work done under contract with the Office of the Surgeon General.

179. *A study of the anatomical response of the lymphoid system to adrenocorticotropin.* Burton L. BAKER, Dwight J. INGLE, and Choh Hao LI, University of Michigan, The Upjohn Company, and University of California.

The concept held by some investigators that the breakdown of lymphocytes serves as a source of serum proteins and antibodies seems to be incompatible with the many reports of failure to observe these results in animals or man treated repeatedly with adrenocorticotropin or adrenal cortical steroids. Therefore, we have studied the thymus, spleen, and lymph nodes of rats which received adrenocorticotropin for variable periods of time and at varying doses in order to evaluate the capacity of the lymphoid organs to produce lymphocytes under these conditions. The following conclusions have been reached. The adrenal steroids induced the breakdown of lymphocytes and thymocytes. However, when treatment was continued for several days the parenchymal cells were depleted and the microscopic picture became one of failure to produce new lymphocytes or thymocytes. Thus, it may be inferred that lymphoid organs could not continue to serve as a significant source of either serum proteins or antibodies. The thymus and lymph nodes were more sensitive to the action of adrenocorticotropin than was the spleen. The highest doses of adrenocorticotropin employed (6 mg daily for 21 days by continuous injection) caused a profound involution of the spleen in which the sinusoidal system apparently had ceased to function. In two of these spleens generalized hematopoiesis was induced in the red pulp.

118. *Leiomyomas of the uterus in the hamster following treatment with diethylstilbestrol and testosterone.*¹ Robert L. BACON, Department of Anatomy, Stanford University.

As part of an investigation of endocrine factors in the production and control of renal tumors in hamsters, a group of 10 females was treated with stilbestrol and testosterone. Beginning on the 50th day of life each animal received 0.6 mg of stilbestrol and 1.0 mg of testosterone base in saline suspension every other day. After from 82 to 89 days of treatment, injections were discontinued and each animal was implanted subcutaneously with a 20 mg pellet of stilbestrol and a 30 mg pellet of testosterone propionate. The animals died or were sacrificed from 223 to 400 days after treatment was started. In addition to the cystic glandular hyperplasia encountered in all uteri, multiple leiomyomas in

one or both uterine horns were found in 6 of the 10 animals. The tumors were positive to tests for alkaline phosphatase but negative to those for acid phosphatase and lipase. Of 20 females treated for equal or greater lengths of time with stilbestrol alone, first in oil and later in saline suspension, a single tumor nodule developed in the uterus of each of 2 animals. These were leiomyomas, but presented a somewhat different histological picture. No uterine tumors have been found in any untreated females in our colony.

Thus the hamster differs from other species in which testosterone has been reported to prevent the development of uterine tumors.

¹ These experiments were supported by grants from the American Cancer Society and the National Cancer Institute.

92. *The influence of injection site on the segregation of acid colloidal substances.*

Ralph N. BAILLIF, Department of Anatomy, Tulane University School of Medicine.

Non-excretable colloids (Chlorazol black E, mercuric sulphide or thorotrast) were injected subcutaneously, intravenously or intraperitoneally into rats. After varying periods the segregation pattern was studied by histologic, and following the use of thorotrast, by radiologic methods.

Small subcutaneous injections of colloid are segregated primarily by histiocytes within the tissue adjacent to the injection site. Larger injections cause abscess formation; the fluid centers of these masses are apparently retained indefinitely. In no case was there sufficient systemic spill-over to produce collections of loaded macrophages in other parts of the body.

Repeated intravenous injections are followed by gradual accumulation of the material in macrophages throughout the loose connective tissues and in walls of the sinusoids (liver, spleen, adrenal cortex and hypophysis). Lymph nodes and thymus are practically free of the colloid.

Repeated intraperitoneal injections produce copious segregation in mesenteric and mediastinal nodes, and in histiocytes of the omentum, mesentery and peritoneum proper. Only after several months of daily injections do loaded macrophages appear in the visceral sinusoids. Colloid was never encountered in cervical or inguinal nodes following intraperitoneal injections.

These findings indicate that there is a remarkably complete degree of segregation by phagocytes in immediate contact with the fluid (tissue juice, lymph or blood) bearing the injected material. There is but little drainage of the colloid from one pool to another except after protracted dosage.

127. *The effects of several new antithyroid compounds on the thyroid and pituitary glands of the albino rat.* W. H. BARRETT, Department of Biochemistry, University of Kansas, G. L. ELLIOTT and E. L. HOUSE, Department of Anatomy, New York Medical College.

A colony of 40 rats, 20 males and 20 females, ranging in age from 6 to 8 weeks, was divided into 4 groups of 10. Each animal in the first three groups was given daily doses of the drug dissolved in distilled water and administered by intubation. The fourth group was intubated daily with an equal volume

of plain distilled water. Additional animals were given comparable doses in food or drinking water. The rats were weighed frequently. Generally, all animals were chloroformed (24 hours) after the 22nd dose. Thyroids and pituitaries were immediately extirpated. The former were weighed and fixed in 10% formalin, while the latter were fixed in Zenker-formol. The thyroids were imbedded in paraffin, sectioned at 5 to 10 micra and stained variously. The pituitaries were weighed after fixation, imbedded routinely, sectioned serially at 4 to 5 micra and stained by a modified Mallory technique. Cell counts of every 15th section of each series were made. Although the growth curve of the experimental animals approximated that of the controls, a definite increase in the average thyroid index was observed. A wider range of variability was also noted in the experimental groups. A marked diminution in colloid content together with hypertrophy of follicular cells was seen. No appreciable changes appear in the average pituitary weights. Some cellular differences were demonstrated.

198. *On the cytochemistry of motor neuron differentiation.* D. H. BARRON and N. Karle MOTTET, Yale University School of Medicine.

That motor neuron differentiation in the anterior horn of chick embryo spinal cord is regulated by the selective induction of indifferent mantle cells has been demonstrated by cytochemical methods. From 63 chick embryos the right posterior limb bud and adjacent axial tissue (the periphery) were removed before three days' incubation. The contralateral (left) side was the control. Employing Feulgen, Nissl, and Gomori reactions the desoxyribonucleic acid, ribonucleic acid, and acid phosphatase, respectively, were studied in the spinal cord of embryos from 4 to 15 days' incubation. In the controls the nuclear desoxyribonucleic acid reaction was diminished (beginning during the fourth day of incubation) in cells differentiating into motor neurons. Concomitantly, the cytoplasmic ribonucleic acid and acid phosphatase reactions became more intense. These cytochemical changes parallel the morphological differentiation— increase of cytoplasmic volume, axonal growth. On the affected side the anterior horn cells that normally differentiate into motor neurons remain cytochemically indistinguishable from mantle cells. Similar observations were made on the associated spinal ganglia. The observations support the morphological evidence demonstrating the regulation of neuron development to be a process of selective induction by the periphery of indifferent cells to differentiate into neurons. Furthermore, they show the concentration of at least some of the chemical constituents of neurons to be regulated by the peripheral field (not intracellularly).

15. *The aortic arch derivatives in the human adult.* Alexander BARRY, Department of Anatomy, University of Michigan Medical School.

One of the traditional phases of embryological teaching involves the fate of the embryonic aortic arches. The conventional diagrams, based on those of Rathke, indicate fairly adequately the general way in which the retention or regression of the various components of the embryonic aortic arch system result

in the establishing of the normal and most of the abnormal patterns of the arterial stems arising from the heart. However, there is a considerable hiatus between these schematic diagrams and the definitive configuration of the arch of the aorta and its main branches in the human adult. During development there are radical shifts in relationship due to the caudal migration of the heart itself and to the concomitant differential growth of its associated vessels, making the adult configuration somewhat difficult to analyse satisfactorily in terms of its original embryological components.

This communication presents a series of diagrams showing the positions of the segmental components of the embryonic aortic arch system as they have come to lie in the adult. A few typical abnormal configurations are presented in comparison with the normal. It is hoped that these diagrams, keyed as they are to segmental levels, will aid in describing anomalies of the arch of the aorta and its main branches in terms of their probable developmental origin.

170. *The gross anatomy and histology of the digestive tube of the toadfish, *Opsanus tau*.* Thomas BATTY and H. C. TRACY, Department of Anatomy, University of Kansas.

The gut in the adult toadfish is simple consisting of three loops. When the living gut is stroked it shortens four fold. The adult gut in the fixed preparation is two-thirds body length and forms an S-like curve. Distinguishable is a very short esophagus, stomach, small and large intestine. The mucosa when examined grossly shows a definite pattern for the three divisions. Pancreatic tissue with islet cells can be seen around the large mesenteric vessels. Small nodules are seen on the mesenteric vessels and believed to be adrenal tissue.

The esophagus can be distinguished from the stomach by striated muscle. The stomach has an outer longitudinal and inner circular layer of smooth muscle. Loose connective tissue forms greater part of the wall and supports gastric glands, without mucosa muscularis. The mucosa is in large folds with numerous glands and the lumen is lined with simple columnar epithelium. There are no goblet cells in the esophagus or stomach. The small intestine is reduced in both muscular and connective tissue layers. The epithelium is more granular and has goblet cells. Mucosa is highly irregular without glands. Blister-like structures appear on large intestine due to extreme thinning of tissue layers. The anal end of large intestine again resumes a thick wall of muscle and connective tissue, with the mucosa in low folds and the epithelium high pigmented and dotted with goblet cells.

190. *The mechanism of tooth formation in the rat as revealed by P^{32} radioautographs.* Leonard-F. BÉLANGER, Department of Histology and Embryology, University of Ottawa.

The fate of a tracer dose of radiophosphate in rats has been studied, using the inverted autograph technique. The radiophosphate is localized in the dentine of the 2 day animal as a sharp line situated at a short distance from the odontoblasts. This line ascends rapidly towards the dentino-enamel junction at

4 and 6 days. From the 6th to the 12th day, it seems to move at a much slower rate. In the areas of most rapid growth it disappears at the 10th or 12th day.

In the very young enamel the mineral deposition takes place in the area farthest away from the ameloblasts. This area is apparently subjacent to an acidophilic red line visible with the Mallory stain. As the enamel matures the acidophilic line is displaced upwards by the growth of the underlying basophilic layer; in parts of the 1st molar of the rat of 16 days the line has disappeared and the radiophosphate is deposited throughout. Along the constantly growing incisor a similar process of maturation can be visualized.

In the 50-gm rat both the dentine and the enamel of erupted molars show a considerable decrease in the deposited P^{32} , as compared with the non erupted tooth.

38. *Epithelial metaplasia and alkaline phosphatase.* Howard A. BERN, Department of Zoology, University of California, Berkeley. (Introduced by Morgan Harris)

The Barger-Gomori cytochemical technic for the localization of alkaline phosphatase is useful in studying the process of epithelial metaplasia and may provide information as to the origin of the metaplastic cells. Early evidences of basal cell proliferation can be detected, and the contrast between alkaline phosphatase-inactive original epithelium and alkaline phosphatase-active replacing epithelium is often quite striking. Some observations suggest the possibility of a contribution from the subepithelial stroma in the metaplastic process.

Studies have been conducted principally on male sex accessory and duct tissues, where the cause of the experimentally induced metaplasia has been either vitamin A deficiency or prolonged estrogen treatment. The alkaline phosphate activity of epithelium undergoing squamous metaplasia and cornification due to estrogen has been correlated with the formation of fibrous protein (keratin) by previous workers. However, the non-cornifying "transitionoid" metaplastic epithelium in the rabbit is also positive for alkaline phosphatase activity, and the cornifying epithelium of avitaminotic-A lesions is generally negative. It is suggested that the histologic picture due to avitaminosis-A may resemble only superficially that due to estrogen. Differences and similarities in the metaplastic process in the male genital tract of mice, rats, guinea pigs, and rabbits will be described.

79. *Incomplete hepatic inactivation of hormone produced by the ovary grafted intrasplenically in the mouse.* E. C. BERNSTORF, Division of Anatomy, Hahnemann Medical College. (Introduced by R. C. Truex)

The combined uterus-vagina weights of (1) spayed mice, of (2) spayed mice bearing an autoplastically grafted ovary in the spleen, and (3) of unoperated animals were compared. The organs of graft-bearing mice weighed significantly less than those of the controls, whereas the organs of the castrates weighed

significantly less than those of the graft-bearing mice. This indicates that sex hormone produced by an ovary situated in the spleen is not totally inactivated by the liver. Histological examination of the uterus and vagina from graft-bearing mice supports this conclusion.

The theory of Jungck, Heller, and Nelson (1947), based in part upon the assumption that complete removal of sex hormone occurs in such graft-bearing animals, should be reconsidered in the light of the above new evidence.

51. *The distribution of olfactory impulses.* Charles BERRY, Wilbur HAGAMEN and Joseph HINSEY, Department of Anatomy, Cornell University Medical College.

The cat brain was explored with bipolar recording electrodes of small dimensions to permit discrete localization of activity following single shock stimulation of an olfactory bulb.

The distribution of olfactory impulses was found to be more extensive than previously reported by Fox, McKinley and Magoun ('44). Spikes were located in the lateral olfactory tract, olfactory tubercle, pyriform areas, hippocampus, hippocampal gyrus, amygdala, fornix, anterior commissure, anterior nucleus of the thalamus, mammillo-thalamic tract, stria medullaris thalami, habenular nucleus, and the habenulo-peduncular tract.

Also, spikes were located throughout large areas of the corpus striatum including the claustrum, putamen, globus pallidus, ansa lenticularis, and in areas within or near the stria terminalis from the region of the anterior commissure to the ventral side of the head of the caudate nucleus.

124. *Fertility in gonadotrophin-treated estral and pseudopregnant rabbits.* W. G. BLACK, R. L. MURPHREE, Gladys OTTO, and L. E. CASIDA, Department of Genetics, University of Wisconsin.

The fertilization rate of estral rabbits, ovulated by intravenous injection of gonadotrophin and vaginally inseminated, was found to be 97%. Deposition of semen in the uterus of artificially ovulated estral does by laparotomy resulted in a fertilization rate of 95%. Pseudopregnant does, artificially ovulated and vaginally inseminated ten days after the sterile mating which produced the pseudopregnant condition, had a fertilization rate of only 3%. When pseudopregnant rabbits were ovulated and semen deposited in the uterus by laparotomy, however, the fertilization rate was found to be 61%. Most of the does in the latter group showed pyometra and endometritis when sacrificed. Other estral and pseudopregnant does were sacrificed 15 days after ovulation and uterine insemination. The percentages of corpora lutea accounted for by normal embryos were 35.5 and 0.0 respectively. Equally-cleaved ova obtained from either estral or pseudopregnant rabbits showed equal survival potentialities when transplanted into either estral or pseudopregnant-host does. The failure of the fertilized ovum of the pseudopregnant rabbit to survive *in situ* is therefore not due to defects of the artificially ovulated ovum nor to an imperfect uterine environment, but rather to an inflammatory condition of the reproductive tract resulting from deposition of semen directly into the uterus.

32. *Observations on the morphology of rat spermatozoa mounted in media of different refractive indices and examined with the phase microscope.* Richard J. BLANDAU, Department of Anatomy, University of Washington.

Rat spermatozoa removed from the epididymis and examined with the phase microscope in either the fresh or fixed state show a few details of their morphology. If, however, the sperm are mounted in media of differing refractive indices as recommended by Crossman (Stain Tech., 24, 61, 1949) and examined with the Dark-M objectives of the phase microscope, certain details of the finer morphology may be clearly visualized. The perforatorium extends beyond the nuclear portion of the head as a ventrally directed rod of great optical density. Caudally the perforatorium is divided into two prongs which fit neatly along the rostral and ventral part of the elongated nucleus. The perforatorium is enclosed in a layer of homogeneous material which continues as a covering for the nucleus. The axial filament extends throughout the middle-piece and tail and is composed of 7 to 9 continuous fibrils. The spiral sheath of the middle-piece is completely ensheathed by a homogeneous membrane. Certain details of the formation of the spiral sheath will be discussed. The axial filament of the tail is encased in a ribbon-like sheath which surrounds the filament in a spiral fashion. The fate of the various parts of the sperm in fertilization will be discussed.

84. *Physical and chemical properties of sludged blood.* Edward H. BLOCH, Department of Anatomy, Western Reserve University. (Introduced by Normand L. Hoerr)

Microscopic observations of human circulation to $150\times$ and in animals to $500\times$ reveal that many diseases and physical agents alter, and some visibly coat, erythrocytes. Healthy erythrocytes are single, repel each other, are not phagocytized. Altered erythrocytes stick together to form aggregates which often have strong surface charges capable of attracting similar erythrocytes or erythrocyte clumps over distances of 10 to 30 micra; such cells are phagocytized in liver in some diseases. Erythrocytes in some pathologic processes have thick, measurable, highly refractile coatings, irregular in outline. Heparin and dicumarol interfere with erythrocyte coat formation; heparin prevents destruction of such cells by liver phagocytes (under special conditions). Quinine, atabrine and sulfonamides may modify surface coats and break up aggregates.

Visible coatings on erythrocytes were refractile by dark field microscopy, and could be drawn into long viscous filaments having high tensile strength by microdissection. Microcataphoresis of erythrocytes with altered surfaces gave mobilities similar to fibrin but different from healthy erythrocytes. Plasma protein analysis by Tiselius electrophoresis revealed a new alpha-globulin fraction and sometimes asymmetry of fibrinogen fraction when massive sludge was present. Knowlesi malaria plasmas had refractile, viscous protein precipitates during dialysis.

According to present concepts, sludge formation may be considered as occurring in three phases: Phase I, the release of substances from damaged tissue cells induced by physical agents or disease; Phase II, reactions of these substances with plasma constituents forming precipitates which in Phase III, coat erythrocytes (? coating of leucocytes and vessel walls).

6. *The opossum neurohypophysis: new evidence on nerve endings, histological structure, and lobular organization of the pars nervosa.* David BODIAN, Poliomyelitis Laboratory, Department of Epidemiology, Johns Hopkins University.

The opossum dispels the perplexity produced by sections of pars nervosa of other mammals. Its pars nervosa consists of lobules, outlined by vascular connective tissue septa, and constructed around branches of the hypothalamo-hypophyseal tract. The tract is the hilum of the gland, and its branches, intermingled with Herring bodies and with the nucleated portions of pituicytes, form the hilum of each lobule. Between hilum and septal border of each lobule is a zone poor in nuclei. This zone can be analysed only in well-fixed material stained with silver or with Gomori's chrom-alum hematoxylin method (Am. J. Path., 17, 395, 1941) which Bargmann (Ztschr. Zellforsch., 34, 610, 1949) has shown to reveal the specific material of the hypothalamo-hypophyseal system (Herring substance). Protargol material reveals innumerable, fine, rod-like endings of nerve fibers of the hilum, arranged perpendicular to the septal border of the lobule. The hematoxylin of the Gomori stain selectively stains this zone, as well as Herring bodies in the hilum, and reveals the palisade arrangement of the Herring substance, which appears to surround the nerve fiber terminals in sleeve-like fashion. Interspersed with these elements are phloxin-stained pituicyte fibers which extend toward the septa like typical supporting glia cells. The relation of the Herring substance in the palisade zone to the nerve fibers and the pituicytes seems to be analogous to the relation of the myelin of nerve fibers to the axon and Schwann cells, respectively. Its clear-cut plan of organization makes the opossum pars nervosa admirably suited for histophysiological and histochemical studies.

13. *The distribution of apical bronchi in anomalies of the right upper lobe.* Edward A. BOYDEN, Department of Anatomy, University of Minnesota.

The recent finding of two anomalous lungs in cadavers—one containing a lobe of the azygos vein, and another a tracheal bronchus—has made possible a detailed study of the apical bronchi in such anomalies.

The first specimen confirms Foster-Carter's conclusion (1946) that the extra lobe is a portion of the normal right upper lobe but not a segmental portion; for in this specimen the azygos vein and its pleural mesentery subdivide the upper lobe by separating the more medial from the more lateral rami of the apical (B¹) and the posterior (B³) segmental bronchus. This lends credence to the view that during development the azygos vein either indents the apex or forms a wedge-like obstruction around which an already well-branched tree must grow. However, the explanation that the azygos lobe is due to the failure of

the vein to slide medially over the growing lung apex would seem to be untenable since it is based on the discarded view that the vein represents a persistence of the posterior cardinal vein.

Dissection of the second specimen reveals that the tracheal bronchus is merely a displaced apical ramus (B¹a) and that the eparterial bronchus supplies the rest of the lobe, dividing into a posterior (B³) and an anomalous anterior segmental bronchus (B² + BX¹b). This analysis confirms the wisdom of Foster-Carter in distinguishing between displaced and supernumerary bronchi.

184. *Possible late tumor development in rat embryos irradiated with 100 r of x-radiation on the 9th day of gestation.*¹ Robert L. BRENT and H. Charles JORDAN, Department of Anatomy, University of Rochester School of Medicine and Dentistry. (Introduced by Karl E. Mason)

Rat embryos were isolated in utero and given 100 r of x-radiation. In one series sacrificed at intervals up to and including 15 days gestation, groups of tumor cells apparently originating from neural tissue were observed in the cephalic portion. These tumors vary in size, differentiation, and position; some are associated with brain tissue, others with the skin, and many are distributed in the intervening mesenchymal tissue.

A second series of embryos were subjected to the same procedure and sacrificed at intervals from 15 days gestation until term. Gross observations have revealed tissue masses of such size as to prevent closure of the mid-sagittal suture. The largest of these growths were observed at 15, 16 and 17 days, while growths observed at term are uniformly smaller. This apparent failure of embryos bearing the larger growths to survive may account for the sharply increased incidence of resorption seen at term as compared with 16 days. Histologic studies are in progress to determine whether these masses are identical with the above mentioned types of tumor or are distortions of the brain wall.

¹ Based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

196. *Histologic alterations in the spinal cord of the rhesus monkey produced by intrathecal injections of epinephrine, with notes on neurological symptoms following the injections.* Kenneth R. BRIZZEE, Jone J. WU, Dana-Lee HARNAGEL and Scott M. SMITH, Departments of Anatomy and Anesthesiology, College of Medicine, University of Utah.

Nissl preparations of spinal cord tissues from animals which received single doses of from 0.88 to 2.6 mg/kg of epinephrine in 0.1% solution and were sacrificed 1-2 hours following maximum manifestation of neurological signs showed slight chromatolytic changes in the large motor cells of the anterior horn of the spinal cord. In general, those receiving larger doses showed slightly greater alterations but some exceptions were noted.

In two animals which received 10 injections over a period of 2 weeks totaling 10.0 mg/kg, the changes were widespread throughout the gray matter. A large percentage of cells in these two preparations revealed nearly complete fragmentation of the chromidial substance.

Tissues from animals sacrificed 24, 48, and 72 hours following a single injection of 1.0 mg/kg showed somewhat greater alterations than those taken from animals which were sacrificed 1 or 2 hours following development of neurological symptoms.

Following each injection, the animals showed a rise in blood pressure and an increase in heart rate. In most cases tremor, rigidity and hyperextension of the lower extremities developed followed by some degree of analgesia and muscular weakness. The intensity and duration of these symptoms generally varied directly as the dosage.

55. *Functional restoration of the paralyzed diaphragm following the cross-union of the vagus and phrenic nerves.* James O. BROWN and Victor P. SATINSKY, Daniel Baugh Institute of Anatomy, Jefferson Medical College and the Department of Surgical Research, Mount Sinai Hospital of Philadelphia.¹

The concern here is with the functional outcome following the cross-union of certain peripheral nerves. Both the silk suture and plasma clot techniques were used for suturing the nerves with no detectable differences in the final result. After a length of time more than sufficient to permit nerve re-growth, the cross-anastomosed vagus and phrenic nerves re-activated the paralyzed diaphragm. In our experiments, the functionally restored diaphragm was observed directly by means of the fluoroscopic screen to display active, rhythmic and automatic movements.

¹ Aided by a grant to one of us (J. O. B.) from the Ella Sachs Plotz Foundation.

87. *The effect of a protein deficient diet on the bone marrow of albino rats.*¹ Jerry W. BROWN, Department of Anatomy, University of Kansas. (Introduced by Paul G. Roofe)

In order to study the effects of a protein deficient diet on the bone marrow, nine albino rats were placed on a diet deficient only in protein (0% protein) and nine control rats were given a complete diet (18% protein). After a period of 50 days on these diets, the animals were sacrificed. A pencil of bone marrow was removed from a femur of each rat by means of dental burrs and a rotary saw. After fixation in Zenker-formol, the serial sections were stained with eosin-azure II-hematoxylin. The cells in 5 fields in each section of bone marrow studied were sketched and counted by means of a camera lucida. The number of cells per cu mm of bone marrow of each rat was then computed.

In the protein deficient rats the numbers of hemocytoblasts, reticular cells, and basophils were not significantly altered, while the plasma cells, megakaryocytes, and all forms in the eosinophil, neutrophil, and erythroid series were significantly decreased in number. The erythroblasts were decreased to a greater extent than the normoblasts and the early forms of eosinophils more than the late forms, while in the neutrophil series the more mature forms showed a greater decrease than the younger forms.

¹ This research supported by Office of Naval Research.

128. *Hyperthyroidism and propylthiouracil-induced hypothyroidism and reproduction in female guinea pigs.*¹ Mina MacNair BROWN and Barbara RAYNER, Department of Anatomy, University of Kansas. (Introduced by Homer B. Latimer)

Forty-one female guinea pigs received 100 γ thyroxine/4 days for about 8 months and were mated when found in heat. Oxygen consumption, heart rate, and serum protein-bound iodine (PBI) values were determined for 5 animals (I) a month after weaning their litters and for 5 that had not been mated (II).

Ten females were given approximately 25 mgm propylthiouracil/day in drinking water. After a month they were mated when found in heat. After a year of treatment, oxygen consumption, heart rate, and PBI values were determined for 7 animals.

Ninety-two percent of the experimentally hyperthyroid animals were found in heat; 97% of the matings were fertile; all animals delivered normally. The average thyroid measures for Groups I and II and controls, in that order, were as follows: oxygen consumption, 92.5, 94.5, 58.9 cc/100 gm/hr; heart rate, 334, 332, 265 beats/min.; PBI, 7.3, 10.0, 2.3 γ %, respectively.

Of the propylthiouracil hypothyroid animals 7 were mated 11 times with 90.9% fertile matings. Of the 7 experimentally uninterrupted pregnancies, 6 terminated at the end of a gestation of normal length. Experimental and control values for oxygen consumption were 49.6 and 58.9; for heart rate, 231 and 265; for PBI, 0.33 and 0.89 (contemporary PBI controls).

It is apparent that reproduction in the female guinea pig is possible within a fairly wide range of thyroid activity.

¹ Aided by grants from the U. S. Public Health Service.

81. *Distribution and concentration of chorionic gonadotropin in mother and fetus during pregnancy.*¹ Joyce BRUNER, Department of Zoology, The State University of Iowa. (Introduced by Emil Witschi)

Maternal and fetal tissues and fluids were systematically collected during pregnancy; their gonadotropic content was determined by bioassay. The highest concentration in the maternal body is in the blood; the uterine endometrium has a higher content than other maternal tissues. The fetal body has relatively low concentrations, but they are definitely of the range that may be physiologically effective. The ratio of concentration between maternal and fetal body tissues is from 10:1 to 30:1. From the 11th week until parturition the concentrations of gonadotropin fall off similarly in all maternal and fetal tissues and fluids, but more slowly in the urine. During this period the concentrations are reduced to about 1/25 of their original value. The ratio of concentration between the chorionic placenta (site of production) and the blood remains the same, while that between blood and urine changes during the course of pregnancy. This indicates that the gonadotropin may be destroyed, or eliminated by an other than the urinary pathway.

The pattern of distribution suggests that the chorion releases the gonadotropic hormone into the maternal blood. Secondly some of it passes the "placental barrier" and enters the fetal system across the wall of the chorionic vesicle. It reaches the fetal body after further diffusion through the amnion.

¹ Supported by grants from the National Research Council, Committee for Research in Problems of Sex.

183. *Roentgen suppression of growth and tissue viability.* V. V. BRUNST, E. A. SHEREMETIEVA-BRUNST. (Introduced by F. H. J. Figge) and Frank H. J. FIGGE, Department of Anatomy, University of Maryland Medical School.

In previous papers it was shown that irradiated amphibian limbs retain their viability for long periods of time (several years). While maintaining a normal appearance, the treated parts suffer a complete suppression of growth. The purpose of this study was to investigate how long the tissues of the limb of a mouse can retain their viability after irradiation with a dosage of x-rays heavy enough to suppress growth.

The right hind limb of several 2-day old mice were irradiated with 3000 r. More than one year later, these mice were investigated. The treated limbs with suppressed growth had a normal appearance. Some tissues were almost normal in appearance but others showed great degenerative alterations. The greatest disturbance was observed in certain regions of cartilage. Some cells in spite of great abnormality and damage were, nevertheless, viable and lived for a long period of time (more than one year).

The present investigation shows that, in spite of the great differences between the mouse and urodele amphibians, the treated tissues in both animals were viable for a long period of time. The most important biological effect of roentgen rays is a suppression of mitotic activity and therefore a suppression of growth and development of the treated parts.

76. *Time after ovariectomy as related to amount of estrogen needed to prevent castration hypersecretion in parabiotic rats.* William W. BYRNES, Department of Zoology, University of Wisconsin. (Introduced by Roland K. Meyer)

When an ovariectomized rat is in parabiosis with a female littermate, the ovaries of the intact rat undergo hypertrophy. The administration of estrogen to the ovariectomized rat beginning on the day of parabiosis prevents the hypersecretion of gonadotropes and the ovarian hypertrophy in the co-parabiont. In the experiments to be reported, ovariectomized rats were placed in parabiosis with littermates at 30 days of age and autopsied 10 days later. The ovariectomized partner was injected with alpha estradiol daily for the 10-day period. Some of the rats were ovariectomized at the time of parabiosis and others 10 days before the parabiotic union. Data will be presented which will show that when the rat is ovariectomized 10 days prior to the parabiotic union, 70% more estradiol is required to prevent hypersecretion than when the rat is ovariecto-

mized at the time of parabiosis. It is concluded that after the pituitary has undergone post-castration hypersecretion more estrogen is needed to inhibit the production of gonadotropes than when estrogen replacement is begun immediately after ovariectomy. These data provide the basis for explaining the large and unphysiological amounts of estrogen reported necessary to inhibit gonadotrope secretion in castrated and post-menopausal women.

1. *Re-interpretation of the structure of the cerebral cortex.* Berry CAMPBELL, Department of Anatomy, University of Minnesota.

An approach to the anatomy of the cerebral cortex involving new theoretical interpretation and new methodology has been made in an attempt to arrive at a more operational position relative to making the comparison of brains of psychotic with sane, individual with individual, species with species. The laminar structure is re-examined and the six-layered pattern of Brodmann found to be an inadequate and subjective judgment which colors all interpretation based upon it. A method of describing the pattern of the cortex in quantitative terms involving no logical postulates concerning layeration prior to the derivation of the data has been developed. With the new method, the cortical pattern is described according to a series of carefully chosen parameters (cell frequency, total cellular area, myelin density, etc.) as continuous curves representing all levels from pial surface to medullary boundary. The areal variation in the cortex is shown not to support the parcellation concept. Instead, the cortex is found to be a continuum exhibiting a rather simple but meaningful pattern of gradients of architectural structure. This leads to a map of the cortex of entirely different nature than those hitherto drawn. On this map the central fissure, the edge of the striate area, and the rhinal fissure are shown to be regions of steep gradient. Large areas of the frontal, parietal, and temporal regions exhibit low, but patterned, gradients. A detailed inquiry into the structure of the frontal lobe is presented and suggestions are made for the adaptation of familiar terminology to new interpretation.

175. *Influence of pituitary on adrenal chemodifferentiation in the chick embryo.* J. F. CASE, Department of Biology, The Johns Hopkins University. (Introduced by B. H. Willier)

Since changes in adrenal ascorbic acid values are indicative of adrenal functional activity, the concentration of this substance has been determined in the adrenals and livers of normal and decapitated White Leghorn embryos in the hope of determining the earliest time at which the anterior pituitary exerts a trophic influence upon the adrenal. By means of the assay procedure of Roe and Keuther (1943), adrenal ascorbic acid of normal embryos was found to rise gradually from 0.85 mg/gm (wet weight) on day 13 to a maximum of 1.75 mg/gm on day 17, from which time there is a gradual decline which continues through hatching to 1.30 mg/gm on the 5th to 10th day post hatching. However, the adrenal ascorbic acid level of 17 day embryos, decapitated at 33 to 38 hours incubation, is only 0.98 mg/gm. That this is a specific effect on

the adrenal is indicated by the fact that liver ascorbic acid levels of normal and decapitated embryos are not significantly different. This effect may be interpreted as indication that the pituitary exerts its adrenocorticotrophic effects at least by the 13th day. Furthermore, the relatively high ascorbic acid levels of adrenals of decapitates argues for a certain amount of initial chemo-differentiation in the absence of the pituitary.

178. *Microscopic changes in the brain following treatment with adrenocorticotropin or cortisone.* C. William CASTOR, University of Michigan. (Introduced by Burton L. Baker)

A study of the histological effects in the brain induced by adrenal cortical hormones is important from two standpoints: (1) to explain the marked changes in personality which are observed frequently in the clinical use of these substances and (2) to locate the center(s) involved in the neural control of adrenocorticotrophic secretion by the anterior hypophysis. Brains of adult male rats injected with 1-6 mg of adrenocorticotropin daily for 21 days or 10 mg of cortisone acetate for 10 days were serially sectioned as far caudally as the posterior commissure and stained with toluidine blue. Adrenocorticotropin caused chromatolysis and reduction in size of the nucleus, nucleolus and cells composing the magnocellular portion of the paraventricular nucleus of the hypothalamus. The effects of cortisone were more widespread, inducing chromatolysis in the paraventricular and supraoptic nuclei of the hypothalamus and chromatolysis and cytoplasmic vacuolation in several nuclei of the thalamus including the N. anterodorsalis, anteroventralis, lateralis thalami, lateralis pars posterior, parataenialis and medialis ventralis. No pathological changes were found in the cerebral cortex. These observations do not define clearly the site in the brain which controls pituitary secretion but they suggest that personality may be modified in the thalamus and hypothalamus.

88. *Lymphocytes in the epithelium of the healthy gingiva.* Martin CATTONI, Department of Oral Pathology, Northwestern University Dental School. (Introduced by Harry Sicher)

Lymphocytes were found to be constantly present in the epithelium of the healthy gingiva of man and dog. In biopsy material these cells were found passing through the basal membrane, entering into the intercellular spaces and pushing aside the cells of the basal epithelial layer. In this position both lymphocytes and basal epithelial cells showed a physical distortion. When the lymphocytes pass through the basal layer their normal shape reappears. Few lymphocytes were found but their presence was constant in all of the specimens studied. The lymphocytes were most abundant in the germinative layer and fewer in the prickle cell layer. The lymphocytes never reach the superficial part of the epithelium. The following changes were observed in the cytoplasm and nucleus of the lymphocytes. The cytoplasm became both wider and clearer while the nucleus stained more deeply. But in some cases this migration was accomplished with slight changes in cell area and morphology. No mitotic figures

were observed among the lymphocytes. All the lymphocytes seen were intercellular in position. The connective tissue underlying the epithelium showed no definite signs of injury. There were no indications of proliferation of lymphocytes or plasma cells.

195. *Intercranial vascular nerves from cranial nerve roots.* Kermit CHRISTENSEN and Elizabeth LEWIS, Department of Anatomy, St. Louis University, School of Medicine.

The origin of intracranial vasomotor and vasosensory nerve fibers from cranial nerve roots has been both affirmed and denied. Whole mounts of the leptomeninges with the cranial nerve roots and the blood vessels from the base of the cat's brain, prepared by the protargol technique and studied under high magnifications, show that nerve fibers from cranial nerves form portions of the plexus along the small blood vessels and in the meninges. The first observations have been made on the VI and XII cranial nerves. Fine, unmyelinated fibers arising from near the ends of the nerve rootlets detached from the brain stem, divide into fibrillae that are the chief components of the plexus. The parts of the plexus that pass along the small blood vessels come from the cranial nerve fibers directly or from the meningeal plexus, and although nerves and blood vessels may be closely associated for a distance, it is not uncommon for the same nervous tissue to leave the blood vessels, take up a new course in the meninges and sometimes terminate there. In true nervous terminations, which are seen rarely, the fibrillae gradually disappear by tapering or by breaking up into minute granules. Ovoid enlargements and minute gemmules scattered along the course of some fibrillae also may serve as terminal structures. The probable function of these vascular nerves is being studied.

134. *Nerves and nerve endings in mouse skin.* C. H. U. CHU, Department of Anatomy, State University Medical Center at New York City. (Introduced by D. M. Kraemer)

Shaved skin of Swiss strain albino mice was macerated with 1% formic acid into four layers. Each layer was stained with periodic acid-leuco fuchsin and mounted in toto. There are two types of nerve fibers, two plexuses and 5 different nerve endings.

Each nerve bundle entering the skin contains myelinated and fine myelinated fibers. In panniculus carnosus, the myelinated fibers terminate at the striated muscles as motor nerve-endings and muscle-spindles. No nerve ending is noticeable in panniculus adiposus. Between panniculus adiposus and dermis, the nerves anastomose to form a plexus of a definite pattern. From this plexus, the myelinated fibers terminate at the hair follicles as end-bulbs and between the hair follicles as vermiform nerve-endings (sensory?). The fine myelinated fibers form a plexus in the dermis immediately beneath the epidermis, sebaceous glands and erector pili muscles as free nerve-endings.

52. *Cessation of spontaneous walking elicited by stimulation of various sub-cortical structures in the unanesthetized dog.* George CLARK, S. E. GOLDBERG and M. G. GOLDBAND.

Electrodes were implanted aseptically at various subcortical locations in a series of dogs, and the animals were tested on the day following operation when they were awake and alert. Stimulation (60 cycle sine wave) at most of these points produced a cessation of spontaneous walking with no apparent loss of postural tone. Often there was some movement, such as turning the head, associated with this arrest. Supraliminal stimulation elicited various complicated clonic movements outlasting the stimulus. These were often so intense and generalized as to be best described as convulsions. The active points were widely scattered in the white matter and included internal capsule, corona radiata, fornix, corpus callosum, etc. Several were located near the border of the caudate nucleus but arrest was not elicited from points centrally located in the caudate nucleus. A similar (?) cessation of spontaneous walking in the cat was termed "suppression" by Ward and the "arrest reaction" by Hunter and Jasper. It is difficult to see how either of these terms can be applied to the response we have observed.

154. *Regeneration of the transected spinal cord of adult cats.*¹ C. D. CLEMENTE, W. W. CHAMBERS, Leon GREENE, S. Q. MITCHELL and W. F. WINDLE, Department of Anatomy, School of Medicine, University of Pennsylvania.

Intraspinal neurons of adult cats and dogs can regenerate after complete spinal cord transections when collagenous scarring is inhibited and a loose reticular and macrophage matrix is substituted in the lesion (Windle and Chambers, *J. Comp. Neur.*: Oct., 1950). Certain bacterial pyrogens help accomplish this condition. To our previous study 67 spinal cats were added, 34 serving as controls for 33 receiving treatment with Pyromen (Baxter) in doses beginning with 20 gamma/kg and increasing during a two week bout to 200 gamma/kg, followed by a two week rest. Ten received only 2 or 3 bouts and 23 received bouts throughout the experimental period.

Some Pyromen-treated animals were killed at various times after transection. Their spinal cords revealed regenerative activity like that previously reported. Nerve fibers grew into a loose matrix of reticular and macrophage cells, some traversing the transection. Controls showed only the usual scar tissue formation. Animals receiving only 2 or 3 bouts of Pyromen followed by long periods of no treatment revealed encroachment of collagenous tissue from the healed pia mater into the loose matrix which resulted in the locking in of regenerating neurons. Animals in which bouts of Pyromen were continued exhibited no latent scar formation. It is in such animals as the latter that long term studies may reveal functional regeneration.

¹ Aided by grants from Baxter Laboratories, Inc. and The Paraplegia Fellowship Fund.

61. *On the structure of contractile elements in the chick amnion.* Leo P. CLEMENTS, Department of Anatomy, and John FERGUSON, Department of Physiology and Pharmacology, The Creighton University.

In the chick amnion we have recorded on drums rhythmic contractions from strip preparations, and have seen contractions arise alternately in localized centers of pieces free in isotonic saline. Rate of contractions varies inversely with age e.g., 8.2 per minute in 10-day, and 3.8 in 15-day amnions. The motility is increased by acetylcholine, pilocarpine, ephedrine, morphine, histamine, etc., and decreased by adrenaline and benadryl.

At 4 days there are localized centers of syncytial nuclei in the amnionic mesoderm. At 5 days fibrils crisscross in, and radiate from, the syncytial centers in all directions. At 6 days the fibrils are grouped in definite band-like fibers which are continuous from one syncytial center to another. The centers thus become star-shaped. In the above process the syncytial nature of the complex is retained in that branches and protoplasmic bridges connect the radiating fibers with each other. The star-shaped centers increase in size with age, and range from 10-12 to one or two per mm² at 5 and 14 days, respectively. In cross sections the band-like fibers lie between the flat epithelium and the mesodermal splanchnopleure. The latter, in days 6 to 12, contains many cells rich in granules. In living amnions under the phase microscope the band-like fibers appear dark and homogeneous.

31. *Formation of the acrosome and head cap of spermatozoa in mammals as shown by the periodic acid-fuchsin sulfurous acid reaction.* Yves CLERMONT, Department of Anatomy, McGill University. (Introduced by C. P. Leblond)

The acrosome and head cap of the sperm cell constitute a single system which, by means of the PA-FSA staining technique, can be traced back to the Golgi region of the young spermatid and remains sharply separated from other structures throughout spermiogenesis. This may be clearly seen in the guinea pig, the spermiogenesis of which will be described in 4 stages.

In the first or *Golgi stage* the newly formed spermatid contains a slightly stained spherical body, the idiosome, located near the nucleus. Later, strongly reactive granules appear in the idiosome and fuse into one large acrosomic granule. During the second or *cap stage*, the head cap develops out of the acrosomic granule and extends over one pole of the nucleus. The acrosomic granule persists over the head cap while increasing in size. The third or *acrosome stage* is characterized by an enlargement of the acrosome granule to form a solid cone, the tip of which is implanted in the Sertoli cytoplasm. In the final or *sperm maturation stage* the acrosome cone flattens and transforms into a crescent placed on top of the nucleus which has flattened into a disc-like structure.

In the other species examined, the same four stages are easily recognized, the first two being always as described above. In the last two stages, the shape of the acrosome, head cap and nucleus varies widely from species to species.

203. *The paranucleoli (nucleolar satellite bodies) in neurons of several male and female mammals, including man.* Rosette Spoerri COIDAN, Department of Anatomy, State University Medical Center at New York City. (Introduced by C. A. Swinyard)

Barr and Bertram (1949) reported that the neurons of female cats contained nucleolar satellites while similar cells of the male cat did not possess these structures or they were poorly developed. This observation led to the possibility of altering the relationship between the presence of these bodies and the sex of the animal by castration.

Paranucleolar bodies were studied in serial sections of spinal cords, stained with the tribasic stain (Spoerri, 1948). The animals in these experiments were mature male and female castrate and non-castrate voles and cotton rats. Studies of the paranucleolar bodies in 100 neurons of both species showed no difference between the male and female, castrate or non-castrate animals.

These bodies were further studied in the spinal cord in both sexes of a variety of normal mature animals; namely, the cat, albino rat, albino mouse (C.F.W. strain) and white-footed jumping mouse. They also were observed in neurons of the brain stem and spinal cord of male and female Rhesus monkeys and man. Paraffin and celloidin sections stained with the tribasic stain, as well as with cresyl violet alone, were used in the latter study.

It has not been possible to differentiate the sex of the animal on the basis of the paranucleolar bodies for they have been found to be identical in the neurons of both sexes in all species studied.

151. *The distribution of fiber degeneration after experimental lesions in the rat brain.* C. Murphy COMBS, Department of Anatomy, Northwestern University Medical School.

Forty-five albino rats were subjected to hemidecortication both with and without striatectomy or striatopallidectomy. The brains were stained with Cajal, Marchi, Weil and Nissl techniques after varying survival times.

The nucleus anterodorsalis of the thalamus has strong corticothalamic projections but few thalamocortical ones. The anteroventralis projects almost entirely to the cortex but receives few cortical fibers. The anteromedialis remains almost normal after hemidecortication. The medialis has strong cortical connections. The submedius has heavy thalamocortical but weak corticothalamic connections. The ventralis medialis is predominantly thalamocortical. The ventralis dorsomedialis has the strongest corticothalamic connection of all the thalamic nuclei and many of its cells project to the cortex. The ventralis ventrolateralis gives off a stronger thalamocortical projection than the dorsomedialis but receives more extracortical fibers. The parafascicularis has few cortical connections. Reunienis is strongly thalamocortical and receives few cortical fibers. Reticularis does not project to the cortex. The medial geniculate body and the dorsal part of the lateral have corticothalamic and thalamocortical connections. The subthalamic nucleus, substantia nigra and hypothalamus have cortical connections.

The pallidum is connected to: all anterior thalamic nuclei, paratenialis, medialis, submedius, ventralis medialis, parafascicularis, rhomboidalis, ventralis dorsomedialis and ventrolateralis, paraventricularis pars rotundocellularis, subthalamie nucleus, substantia nigra and zona incerta.

The striatum (caudate + putamen) appears to be connected mainly to the pallidum.

168. *Further comparative studies on normal and avascular bone, including density determinations with use of radioactive strontium-90 and the Geiger-Muller counter.*¹ Carl C. COOLBAUGH, Department of Anatomy, Wayne University. (Introduced by F. Gaynor Evans)

Comparative stress-strain analyses have been made upon normal and partially avascular dog femurs. The avascularity of the bone has been produced in varying degrees of severity by three different surgical techniques, the first consisting merely of femoral artery ligation, the second of nutrient artery ligation plus a periosteal decapsulation, and the third, nutrient artery ligation and periosteal decapsulation plus a metaphyseal vascular interruption. In each case the avascular bone when compared to the normal showed a definite increase in the modulus of elasticity. Further comparison was then carried on by determining the bone-densities of the normal and avascular samples. This was accomplished by using the beta-ray emitter source, radioactive strontium-90 and the Geiger-Muller tube. Further comparative studies consisting of chemical analyses of the inorganic constituents of the samples will be reported later.

¹ This research was supported (in part) by a research grant from the National Institutes of Health, U. S. Public Health Service.

12. *Fascias of the back.* George W. COOPER, Department of Anatomy, Louisiana State University School of Medicine. (Introduced by Charles M. Goss)

Fascial layers in the paramedian thoracic areas were found to be ten in number, including vaginal sheaths. Those anterior to the semispinalis muscles were not studied. Minor fascial planes are not amenable to interpretation in terms of large sheets which might form extensive barriers in pathological processes.

The paramedian fascial layers are readily differentiated in the thorax near the midline, but not in the latter, nor are all of them discernible caudad to the rhomboid muscles. There are fewer layers also cephalad to the core fascias (those in paramedian thoracic areas) and in the lateral subcapsular region, as well as in the lateral thoracic and lumbo-sacral areas.

The more numerous planes of the paramedian thoracic regions are integrated by continuity into the fewer layers of the loci cited above.

These fascias were found to be inadequately treated in standard texts and there is discrepancy between some of their descriptions and our observations (1) The extension of the lumbar aponeurosis cephalad to the sixth thoracic vertebra was found at variance with most texts. (2) The lumbodorsal fascia was observed to split at the caudalmost origin of the splenius cervicis, an anterior

and posterior layer surrounding the muscle on these surfaces. (3) These reflections were projected cephalad around the splenius capitis. (4) The semi-spinalis capitis fascia thins progressively as it ascends but thickens markedly upon emerging from the splenii.

41. *The distribution of succinic dehydrogenase and cytochrome oxidase in the nervous tissues of the dog.* S. J. COOPERSTEIN, Department of Anatomy, Western Reserve University. (Introduced by Arnold Lazarow)

Using ultra-microspectrophotometric methods involving measurement of the rate of oxidation or reduction of cytochrome *c*, the cytochrome oxidase and succinic dehydrogenase contents of spinal ganglion, grey matter (anterior horn), white matter, and spinal nerve of dogs were determined. The cytochrome oxidase of grey matter was approximately twice that of spinal ganglion, four times that of white matter, and nine times that of nerve. Grey matter also had twice as much succinic dehydrogenase as ganglion, but none could be detected in nerve and white matter. Considering the sensitivity of the method and the amount of cytochrome oxidase in these tissues, succinic dehydrogenase should have been detected in white matter and nerve even if the ratio of this enzyme to cytochrome oxidase were only 1/2 to 1/5 as great as is the case in grey matter and spinal ganglion. Further studies indicate that the results are apparently not due to an inhibitor in dog nerve and white matter nor to the use of anesthesia (nembutal).

Although this surprisingly low (or absent) succinic dehydrogenase activity was also found in rat sciatic nerve, the squid giant axon and axoplasm contained significant amounts of this enzyme. The dissociation of cytochrome oxidase and succinic dehydrogenase in rat and dog nerve is unusual, since both enzymes generally occur together in tissues. The possible significance of this dissociation will be discussed.

206. *Post-mortem changes in the neurons of the cat spinal cord and cerebral cortex.* Sam CORNWELL, Departments of Anatomy and Neurology, University of Minnesota. (Introduced by Berry Campbell)

Cats were subjected to unilateral femoral and sciatic nerve section and killed ten to fifteen days after operation. Blocks were taken from the lumbosacral enlargement of the spinal cord and from the frontal and parietal cerebral cortex immediately before death and at 6 intervals after death: 20 minutes, 40 minutes, 80 minutes, 4 hours, 10 hours, and 20 hours, respectively. The blocks were then fixed in 10% formalin, dehydrated in dioxan and acetone, embedded in paraffin, and sectioned at 5 and 10 micra. The sections were stained in cresyl violet and erythrosin.

Postmortem changes in the normal and chromatolytic anterior horn cells of the spinal cord and in the pyramidal cells of the cerebral cortex are described and demonstrated. Postmortem alterations in normal cells include: (1) swelling and rupture of the cellular membrane, (2) changes resembling chromatolysis, (3) diffuse basophilia and reticulation of the cytoplasm with occasional extrusion of the

cytoplasm and "ghost cell" formation, (4) diffusion of the nuclear chromatin and formation of a more opaque, homogeneous nucleolus, (5) the appearance of tortuous apical dendrites, and (6) a striking condensation and hyperchromatism of glial nuclei.

192. *The preparation and distribution of iodo-prolactin labeled with I^{131} .* Phoebe L. COX, Department of Anatomy, McGill University. (Introduced by C. P. Martin)

The protein hormone, prolactin, has been iodinated to the extent of converting, on the average, one tyrosine radical per molecule into either the mono- or di-iodo form. Such a preparation has been shown to be only slightly less biologically active than its non-iodinated control. It has been possible to free this material of contaminating iodide to the extent of 99.7% by means of washing and reprecipitation with its isoelectric acetate buffer. When such radio-iodo-prolactin is added to liver homogenate, 97% has been recovered in the precipitate with the aid of the Somogyi zinc sulfate reagent used for the determination of protein bound iodine; and by the same procedure all but 2% of a similar quantity of labeled iodide has been removed from the same fraction.

0.1 mgm of such a partially iodinated radioactive prolactin was injected intravenously into C_3H mice and its distribution in the tissues and organs was studied at various time intervals. At 20 minutes following intravenous injection the highest concentrations of labeled protein have been found in the liver and kidney. Appreciable amounts of radioactivity were present in mammary glands and milk, as well as adrenal and ovary. The results also indicate that radio-iodo-prolactin promptly leaves the circulation and undergoes a rapid breakdown.

35. *Modification of Regaud's fixative for use in studies with the electron microscope.* Albert J. DALTON, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

Previous trials had shown that for the study of thin sections of tissues under the electron microscope, fixatives containing osmic acid produced results superior to the many others tried. However, fixation of nervous tissue was unsatisfactory because of the presence of large amounts of lipid. In addition the poor penetrating power of osmic acid required fixation by perfusion, and it was found difficult or impossible to perfuse such organs as spleen, testis and ovary, as well as transplanted tumors. Thin slices of tissues fixed by the Regaud method were transferred in graded steps to 95% alcohol to remove the alcohol soluble lipids. These slices were then returned in graded steps to water and from these, still thinner slices were placed in 2% osmic acid at room temperature for 4 hours. Tissue thus prepared is reasonably well fixed, is relatively fat free and does not appear to be altered by exposure to the electron beam. Electron micrographs of several tissue cell types prepared by this method will be shown.

131. *Thyrotrophic hormone content of blood in starvation.*¹ Savino A. D'ANGELO, Department of Anatomy, Jefferson Medical College.

These experiments were undertaken to test one aspect of the hypothesis that in starvation the histo-physiologic changes in target endocrines are referable to effects on trophic mechanisms in the adenohypophysis.

Adult male rats (300–450 gm) and mice (25–35 gm) were subjected to acute starvation by withdrawal of the normal laboratory ration. At 30–35% body weight loss the starved animals, and fed controls, were anesthetized, blood withdrawn by cardiac puncture, and various endocrine organs removed and prepared for histologic study. Bloods of animals in each group were pooled and the raw sera subsequently bioassayed in the stasis tadpole for thyroid stimulating hormone (TSH).

TSH was detected in appreciable and approximately equal concentrations in sera from normally fed rats and mice. Six injections (0.05 cc daily, alternate days) elevated thyroid cell heights in test tadpoles from 5.2 micra (control) to 6.9 and 7.0 micra for rat and mouse blood, respectively. Acute starvation led to significant decrease in blood TSH. Thyroid cell heights with sera of starved rats and mice fell to values of 5.6 and 6.0 micra, respectively. The decrease in blood TSH could be correlated with histologic changes in the thyroid indicative of reduced activity.

The results are considered to present direct evidence in support of the concept that inanition depresses the thyrotrophic function of the pituitary.

¹ Supported by a grant (RG-2407) from the National Institutes of Health.

166. *Relations between the origins of the spinal nerves and the vertebral column.* Ramji DASS and Naranjan DASS, Departments of Anatomy, New York University-Bellevue Medical Center and Medical College, Amritsar, India. (Introduced by Donal Sheehan)

The topography of neural and osseous segments has been reinvestigated by radiological methods on 25 human cadavers, ranging in age from 8 to 35 years. First, lateral radiographs of the intact spinal column were taken in order to ascertain the relative levels of the bodies and spines of the vertebrae. Then, after removal of the arches, pins were inserted transversely through the spinal cord at levels corresponding to the midpoint of the origin of each pair of anterior roots. Next, an antero-posterior radiograph was taken to show the relationships between the neural segments indicated by pins and the vertebral bodies. Elaborations of, and marked deviations from, data given by previous workers will be presented and illustrated. Contrary to Reid's and Chipault's observations, it was seen that the cervical segments are coextensive with not more than the upper 5 cervical vertebrae. Variations in level of the termination of the spinal cord are correlated with the positions of the lower thoracic, lumbar and sacral neural segments.

174. *Changes in the adrenal cortex of rats with experimental hypertension.* Helen Wendler DEANE, Harvard Medical School, and Georges M. C. MASSON, Research Division of the Cleveland Clinic Foundation.

Selye postulates that hypertension results from the pituitary stimulating the release of salt-retaining hormones by the adrenal. Such hormones appear to arise in the zona glomerulosa (Deane, Shaw and Greep, 1949, *Endocrinology*, 43: 133).

To test Selye's hypothesis, rats were made hypertensive by (1) injection of desoxycorticosterone acetate (2.5 mg daily); (2) encapsulation of the kidney; (3) injection of anterior pituitary powder. Some animals in (1) and (2) drank tap water, others 1% saline; those in (3) saline. Frozen sections of the adrenal glands were stained for lipids.

In the control rats receiving tap water (av. blood pressure 104 mm Hg), the glomerulosa measured 40μ in width and contained moderate amounts of lipid; in controls receiving saline (b.p. 108), it measured 30μ and was depleted of lipids. (1) Rats receiving desoxycorticosterone (40 to 111 days, b.p. 186) had narrow (30μ), depleted glomerulosas. (2) Rats with encapsulated kidneys (73 to 120 days, b.p. 161) exhibited enlarged (87μ) glomerulosas with increased amounts of lipid. Saline administration to this type of rat caused no significant changes (35 days, b.p. 149, glomerulosas 65μ), but desoxycorticosterone administration produced shrinkage and depletion. (3) Rats receiving anterior pituitary powder and saline (48 days, b.p. 174) had atrophied glomerulosas.

These facts fail to support Selye's hypothesis, since, while encapsulating the kidneys appear to stimulate the glomerulosa administration of anterior pituitary powder does not.

80. *Some responses of the immature rat uterus to hormonal stimulation with special reference to fat deposition in the luminal epithelium.* Lawrence F. DIETLEIN, Jr., Department of Biology, Harvard University. (Introduced by A. B. Dawson)

Beginning about the 8th day after birth, the luminal epithelium of the rat uterus exhibits a fine basal accumulation of osmiophilic, sudanophilic droplets. Up to the 8th day, fat is restricted to the muscularis. From the 8th day until the first estrus, epithelial fat increases progressively in amount. After the 15th day, the epithelial fat assumes a bipolar pattern, especially in the antimesometrial area. With age, the globules also coarsen considerably.

DCA administered daily from birth, produces an initial accumulation of fat in the mesometrial epithelium as early as the 2nd day. By the 8th day, the entire epithelium exhibits a rather dense and uniform accumulation of mostly bipolar fat. Progesterone produces a comparable effect, except that more fat is deposited.

Treatment of newborn rats with a single dose of estradiol followed by daily progesterone administration, results in the appearance of fine basal fat on the 2nd day, which becomes progressively more abundant and uniformly bipolar in pattern on the 3rd day.

Adrenalectomy (20-day rats) results in gradual and progressive postoperative diminution in amount of epithelial fat.

A positive alkaline phosphatase reaction in arteriolar and capillary endothelium is apparently restricted to the mesometrial area before the 8th day. By the 12th day, capillaries of the entire uterus exhibit a strongly positive reaction.

Further studies, including glycogen, are now in progress.

56. *Some results of anastomosing the proximal ends of nerves cut near their termination to the distal ends of nerves cut near their origin.* Donald DUNCAN and Emmett N. WILSON, University of Texas Medical Branch.

The capacity of neurons in cats and rats to produce axons of much greater than normal length has been investigated further by anastomosing the proximal ends of ulnar nerves cut at the wrist, or facial nerve branches cut as far peripherally as possible, to the distal ends of ulnar nerves cut at their origin from the branchial plexus. The contractions obtained in muscles supplied by the recipient ulnar nerves on stimulation of the donor nerves demonstrated that the capacity for growth in axon length is much greater than normally occurs, and that such over-extended axons have the capacity to make functional connections in denervated areas.

27. *Influence of testis and thyroid hormones on the epidermis of the male albino rat.* Heidi EARTLY and B. GRAD, Department of Anatomy, McGill University. (Introduced by Z. Menschik)

In a number of organs the combined action of testosterone and thyroxine is necessary for the maintenance of normal histology. These hormones may act synergistically as in the case of the heart, kidney and submaxillary gland or antagonistically as is reported here in the case of the skin.

Male rats which had been castrated and thyroidectomized received replacement doses of testosterone or thyroxine, or both, for 47 days. On autopsy, corresponding pieces of skin were taken from the lumbar region of the animals, fixed in Orth, sectioned and stained with hematoxylin-eosin. A cursory examination of the slides revealed a marked difference in the thickness of the epidermis corresponding to the various treatments. A precise histometric method was then used to permit statistical analysis. The technique consisted of projecting the sections on sheets of paper and then tracing the outlines of the Malpighian and cornified layers. Six fields were chosen at random for each section, and each outline was cut out and weighed separately.

Results showed that testosterone significantly increased the thickness of the epidermis, especially that of the cornified layers, while thyroxine decreased the thickness. When both hormones were given, an intermediate effect was obtained, as if one hormone cancelled the other. The picture obtained resembled somewhat that found in the control animals, thus indicating that a balance of the two hormones is necessary for a normal skin thickness.

18. *Functional anatomy of the porto-caval communications.* Edward A. EDWARDS, Department of Anatomy, Harvard Medical School.

The porto-caval communications were studied, by roentgenography and dissection after caval injection with a barium sulphate suspension, in three post mortem subjects with normal portal systems, and in one with portal cirrhosis. In all, the injection fluid filled most of the portal system via the anastomoses existing in the pelvis, across the retroperitoneal surfaces of the abdominal viscera, and in the mediastinum. The anterior parietal (peri-umbilical) channels were uninjected except in the patient with cirrhosis. The present findings thus confirm the observations of 18th century anatomists of the normal patency of the porto-caval communications.

The deep pathways are theoretically and actually of greater significance than the anterior parietal ones. Normally, the deep anastomoses are numerous and patent, occasionally including veins of intermediate, or large size. Since the communicating veins infrequently possess valves, they are able to conduct blood equally readily in either direction. The communications in the retroperitoneal areas are of greater importance than is usually realized.

The adequacy of the collaterals in obstruction of the portal system is of practical interest. Anatomically, special importance attaches to the anastomoses in the region of the umbilicus and falciform ligament, the spleen, the left kidney and the rectum.

The utilization of the porto-caval communications in the collateral flow after hepatic vein or vena caval obstruction will be briefly discussed. Although the portal and caval patterns of enlargement of the superficial veins of the trunk almost invariably show distinguishing features, a case is presented illustrating the production of a portal pattern following obstruction of the inferior vena cava.

138. *Vascular anatomy of the human liver.* Hans ELIAS and David PETTY, Department of Anatomy, The Chicago Medical School, and Hektoen Institute of Cook County Hospital.

Since the time of Glisson (1659-72) the gross anatomy of the major vessels in the human liver has not received sufficient attention.

By means of the corrosion method the question was reinvestigated.

The portal vein divides into two major trunci. The left trunk supplies the pars umbilicalis hepatis which includes the left lobe, the quadrate lobe and the territory located cranioventrally from it, as well as half of the caudate lobe. The right trunk supplies the territory to the right (pars omphalomesenterica hepatis). Both portal territories are sharply separated from one another as shown by McIndoe and Counseller ('27). The portal branches of the second order are mapped and named. Each has its own, sharply defined territory. There are no portal anastomoses connecting the territories of the first, second, third and fourth order in normal livers. The liver is thus divided into a number of constant, portal segments. These vary in size in various individuals. Individual variations of the points of origin of the rami occur.

The hepatic veins and their major radicles possess equally sharply defined territories. However, the portal and hepatic territories overlap in such a manner that the liver of man presents a unified whole.

The vascular pattern and the relative sizes of the so called lobes of adult livers are explained on the basis of embryology.

161. *Pressure measurements in perfused segments of small blood vessels.* Victor M. EMMEL and Durwood J. SMITH, Departments of Anatomy and Medicine, University of Rochester School of Medicine and Dentistry.

A technique has been developed for continuously recording pressure changes occurring in isolated segments of small blood vessels during perfusion with vaso-active agents. The essential feature of the method is that the perfused specimen is mounted between cannulae within a closed, fluid-filled chamber to which is connected an electrical pressure transducer. An increase in vascular tone will then cause a "negative" pressure in the surrounding chamber, while the intraluminal pressure (i.e., the perfusion pressure) remains constant. Since the diameter of the vessel cannot change under these circumstances, the response of the vessel represents an isometric contraction of the smooth muscle in its wall. On the basis of the vascular diameter and pressure differential, the tension in the vascular wall can easily be calculated.

The method can be applied to vessels of any size, but is particularly useful in studying small vascular biopsies such as obtained from the dog's mesentery. A normal vessel about 1 mm in diameter and 8 mm in length may develop a pressure differential of as much as 300 mm Hg when stimulated briefly with epinephrine at a concentration of 5 ppm. A barely perceptible response occurs at a concentration of 0.1 ppm. The method is being used in studying vascular biopsies from dogs with experimental vascular disease.

132. *The effect of tobacco smoke on endocrine glands of albino mice.* J. M. ESSENBERG, Department of Anatomy, The Chicago Medical School.

The 36 experimental mice were housed in the chamber of a smoking machine which delivered the smoke of one cigarette per hour for 12 hours daily except Sundays. The chamber was filled with smoke during the burning of the cigarette but began to clear soon after. At all times the mice were abundantly supplied with fresh air. The control mice were treated similarly except for the smoke. The experiment lasted 16½ months.

The endocrine glands studied were the pituitary, the thyroid, the parathyroids, the pancreas and the suprarenal glands. The condition of the gonads has been reported previously.

No deviation from the normal could be demonstrated histologically in any part of the pituitary gland or the parathyroid gland. It was different with the thyroid gland. The disappearance of the colloid and the breaking up of the follicles occurred in practically all of the smoked mice but seldom involved the entire gland. Cysts were also noted in some of the thyroid glands. The pancreatic islets seemed less in number with smoked mice. The pathology of the suprarenal

gland was more severe than any of the endocrine glands studied. It varied from a slight effect of the pars reticularis to a total lack of the pars reticularis, pars fasciculata and part of the pars glomerulosa. In many instances the medulla of the suprarenal was enlarged but otherwise presented no pathology.

169. *Some physical properties of the compacta of the human tibia and fibula.*¹

F. Gaynor EVANS and Milton LEBOW, Departments of Anatomy and Engineering, Wayne university.

Standardized samples of compacta from the femur, tibia and fibula of a 47 year-old white male and a 74 year-old Negro male were tested for tensile and shearing strength. In all bones the average tensile strength exceeded the shearing strength. The tibia was the strongest bone in both tension and shear. The femur was the weakest except in one bone in which the shearing strength was greater than that of the fibula of the same individual. The middle third of all bones but one was the strongest. The lateral quadrant of the tibia has the greatest tensile strength and the medial the least. The corresponding quadrants for shearing strength are the medial and anterior, respectively. The various thirds of the tibia showed a greater variation in strength than do those of the femur. However, there is less variation in the strength of the various quadrants. The fibula was not studied according to quadrants. Tests on other tibias gave similar results. In all 131 samples were tested in tension and 125 in shear. All samples were wet. The results of studies of the density of the samples, determined by use of a radio-active beta ray emitter (strontium 90) and Geiger counters, as well as the fatigue strength of bone will also be reported.

¹ This research was supported (in part) by a research grant from the National Institutes of Health, U. S. Public Health Service.

123. *Effects of estrogen-progesterone synergy on thresholds and timing in the "LH-release apparatus" of the female rat.* John W. EVERETT, Department of Anatomy, Duke University School of Medicine.

The LH-release apparatus in female rats is consistently blocked by atropine or barbiturates administered about 2 P.M. during proestrus (4-day cycle). Repetition of barbiturate sedation at the same critical hour will block the ovulatory mechanism day after day. However, after the first day such treatment is effective only when the dose is increased or when multiple injections are given. A possible explanation is a modification of thresholds or of some other attribute of the timing mechanism by more prolonged action of ovarian steroids.

This possibility was tested by giving estrogen (50 µg estradiol benzonate) to 4-day cyclic rats at noon on diestrus day 2 and/or progesterone (1 mg.) at noon on the day of proestrus. Nembutal (30 mg/kg, intraperitoneally) or atropine sulphate (700 mg/kg, subcutaneously) were then given as blocking agents at 2 P.M.

The 2 steroids in sequence caused ovulation in 5/5 rats in spite of Nembutal. By contrast, 11 rats given estrogen only and 5 rats given progesterone only were all blocked by identical Nembutal treatment.

In 7/9 rats the estrogen-progesterone sequence apparently caused pituitary activation somewhat in advance of the usual hour. In spite of the 2 P.M. injection of atropine, a long-term blocking agent, 5 rats ovulated normally and 2 were only partially blocked.

These results further attest the synergistic action of estrogen and progesterone in sensitizing the LH-release mechanism in this species.

191. *The rate and pathway of sulfur excretion in the rat following intravenous administration of S^{35} sodium sulfate.* Newton B. EVERETT and Barbara S. SIMMONS, Department of Anatomy, University of Washington.

It has been demonstrated previously (Hanahan and Everett, '50) that the rat excretes sulfate, liberated from subcutaneous or intravenous S^{35} sodium estrone sulfate, in the urine and feces. Although 10% of the sulfate appeared in bile, a significant quantity reached the feces by other than the biliary route. The present study is primarily concerned with the route of sulfate entry into the feces of bile fistula animals.

Adult female rats, after the preparation of the bile fistulas, were given single intravenous injections of S^{35} sodium sulfate. After intervals ranging from 1 to 24 hours, determinations were made for the amount of S^{35} in the blood, urine, bile and feces, and for the amount present in several segments of the gastrointestinal tract. In addition, the pancreas, salivary glands, tissue fluid and various other tissues were assayed for S^{35} at some of the early intervals.

One hour after the injection, about 7% of the sulfate was found in the upper third of the small intestine. After 10 hours the S^{35} had moved through the small and large intestine and began to appear in the feces. After 24 hours, about 75% of the activity was recovered in the urine, 10% in the bile, and about 9% in the feces and lower intestine.

It is suggested that sulfate enters the upper small intestine directly from the blood or from the tissue fluid.

34. *Parabiosis intoxication.* John C. FINERTY and Theodore C. PANOS, Departments of Anatomy and Pediatrics, University of Texas Medical Branch.

A condition which is peculiar to the parabiotic state interferes with good survival of some pairs. It has been described, primarily in the German literature, and has been referred to as "Parabiose Vergiftung," parabiosis intoxication, as well as by other terms. Most experimental evidence suggests that it is a constitutional incompatibility which expresses itself as an anaphylactoid reaction.

The present work includes a description of the symptoms, discussion of the incidence of parabiosis intoxication and reports an attempt to control it. Seventy one pairs of 31-day old, non-littermate, male rats of the Holtzman strain were joined in parabiosis and observed daily. At the end of 7 weeks of parabiosis 23 (33%) of the pairs were alive in good condition. Nine pairs died as a result of the operative procedure, and 39 (55%) died from parabiosis intoxication. During the period of observation only 4 pairs were completely asymptomatic; 19 pairs showed symptoms of intoxication, but recovered.

Constant indications of intoxication are hyperemia of one partner, anemia of the other and progressive differences in size. The anemic partner is consistently smaller, becomes sluggish and moribund about a day preceding death, and always dies first.

Administration of an antihistamine during the period when symptoms of intoxication appear (7th to 21st post operative days) has no influence on incidence or severity of the intoxication.

82. *Plasma progesterin levels during pregnancy in women and monkeys.* Thomas R. FORBES, Department of Anatomy, Yale University.

Blood samples (1 cc) were obtained at weekly intervals from two pregnant women during the second and third trimesters and from three rhesus monkeys throughout nearly all of pregnancy. The plasma in each sample was separated and subjected to bio-assay for progesterin by the method of Hooker and Forbes (1947, *Endocrin.* 41, 158). Concentrations of the hormone, when plotted against time, fluctuated irregularly, rising infrequently to 2.5 $\mu\text{g}/\text{cc}$ plasma in the human samples and to 3.5 $\mu\text{g}/\text{cc}$ plasma in the monkey samples but usually remaining lower. In the case of each donor there was at least one instance when no hormone could be detected in 2 to 7 successive weekly samples. A low level or an apparent absence of hormone was usually followed by an abrupt rise in concentration. The hormone continued to be present until term.

Bound progesterin levels were very low in all cases; frequently, no bound progesterin could be detected.

77. *The role of progesterone in the production of vaginal changes in the female guinea pig.*¹ Donald H. FORD and William C. YOUNG, Department of Anatomy, University of Kansas.

The possibility that progesterone might be involved in the production of cyclic vaginal changes was suggested by observations on sexually maturing and pregnant female guinea pigs and by the difficulty in producing normal cornification with estrogen alone. This hypothesis has been tested.

Ovariectomized females received an initial subcutaneous injection of estradiol benzoate, a larger injection 24 hours later and a still larger dose on the 48th hour. The total amount ranged from 175 to 600 I.U. Smears were made on the 71st hour and every three hours thereafter until the 95th hour except the 92nd hour.

Full cornification was produced in 50% of the animals only when 200 or more I.U. were given. When, however, such animals received 0.2 I.U. progesterone 24 hours after the last estrogen injection, when the vaginal condition was still proestrous, cornification followed by leucocytic invasion occurred in all animals. Furthermore, the changes developed sooner than in animals given estrogen alone. Progesterone was not necessary for complete cornification when 400 I.U. estradiol benzoate were given, but cornification and leucocytic invasion were accelerated by 0.2 I.U. progesterone.

Apart from the value this information has for work on the functioning of the corpus luteum during sexual maturation and pregnancy in the guinea pig, the addition of this role to the lengthening list of activities displayed by progesterone is of interest.

¹ Aided by grants from the U. S. Public Health Service.

33. *Histological effect of Nitrofurazone in the rat.* Charles E. FRIEDGOOD, C. A. SWINYARD¹ and Charles B. RIPSTEIN, Department of Surgery, Maimonides Hospital and State University of New York, Medical Center.

While studying the effect of Nitrofurazone on the growth of fibrosarcomata of mice one of us (Friedgood, '48, '50) observed adrenal gland hypertrophy and decreased gametogenic activity. Therefore a group of albino rats have been given subcutaneous doses of micronized Nitrofurazone suspended in peanut oil. The total dose given ranged from 0.2 gm to 1.2 gm. In some of the animals given the maximum total dose the liver and kidney showed occasional cystoplasmic degeneration.

The adrenal glands were hypertrophied and vacuolization of the zona fasciculata was accentuated. The gland enlargement was due to fasciculata change. Sudan IV showed an increased lipid content and the Ashbel-Seligman carbonyl stain gave a more intense color reaction in the adrenal cortex.

Gametogenic cells of the seminiferous tubules were markedly affected. In some cases necrosis was initiated with a single 200 mg dose. Four 200 mg doses resulted in total cessation of spermatogenesis. The spermatozoa and more mature gametogenic cells were most sensitive. Sertoli cells were necrotic only at the higher dose levels. Interstitial cells were unaffected.

Several injections of 200 mg produced marked increase in excitability and convulsions. Neurocytological studies and investigations of altered central nervous system excitability are under investigation.

¹ Visiting Lecturer in Anatomy, State University of New York; Professor of Anatomy, University of Utah, Salt Lake City, Utah.

171. *The histology of the rat kidney in post-DCA hypertension.* Sydney FRIEDMAN, Department of Anatomy, University of British Columbia.

Desoxycorticosterone acetate (DCA) was administered to rats in amounts sufficient to produce a marked elevation in blood pressure. It was then abruptly withdrawn. The animals were then followed for over a year after cessation of treatment with frequent estimations of blood pressure and occasional sampling for histological examination of the kidneys.

At the conclusion of the period of active DCA treatment there was considerable hypertrophy of the media in the arterioles and small arteries of the kidney with focal infarction in areas supplied by the most affected vessels. Cessation of treatment was followed by healing, with the previous period of active treatment indicated only by residual parenchymal scars and a minor degree of hypertrophy in the media of the vessels.

About one-third of the treated animals remained hypertensive throughout the period of observation. At no stage, however, was there any anatomical difference between the kidneys of those animals which remained permanently hypertensive and those which became normotensive. It is suggested that the sustained phase of hypertension which follows DCA treatment is similar to essential hypertension. A hypothesis to account for the process is presented.

11. *Cleavage line patterns of the cat.* Joseph H. GARDNER and Harry E. RAYBUCK, Department of Anatomy, Saint Louis University, School of Medicine. (Introduced by K. Christensen)

Pattern formation of cleavage lines and factors which are responsible for these lines have been investigated in the skin of the cat. The cleavage lines were produced by piercing the skin with a sharp conical instrument. The resulting linear wounds were painted black and photographed. By comparing the photographs, consistent cleavage line patterns were observed in the cat. Blocks of tissue containing the linear wounds were removed and sectioned in 3 different planes. Masson's trichrome stain was employed to demonstrate collagenous fibers and histologically, a greater abundance of collagenous fibers are found running parallel with the long axis of the wound. The arrangement of the collagen fibers is responsible for the cleavage line patterns. The patterns do not conform completely to the fiber arrangement of the superficial muscles nor will the epidermis alone give rise to a cleavage line.

188. *A tracer study of the distribution of phosphorus (P^{32}) in metamorphosing tadpoles (*R. pipiens*).* Joseph F. GENNARO, Jr., and J. N. DENT, Department of Biological Sciences, University of Pittsburgh and Miller School of Biology, University of Virginia.

Preliminary to making specific and quantitative analyses of the processes in which phosphorus is involved in the organs of anuran larvae at various stages of metamorphosis it was considered desirable to first examine phosphorus distribution in such larvae. This was done by immersing metamorphosing individuals in a solution of P^{32} (5/ μ c/ml) for periods of 24 hours and then preparing and studying radioautographs made from sections of these individuals. It was found that in general there is a tendency for the P^{32} to be concentrated in organs undergoing morphological change, with the degree of concentration roughly paralleling the rate of change in the skeleton, gut, eye, and horny tooth.

54. *Splanchnic afferent pathways in the central nervous system.* William A. GEOHEGAN and Orlando AIDAR, Department of Anatomy, Cornell University Medical College. (Introduced by Joseph C. Hinsey)

Afferent pathways from the splanchnic nerves in the cat were traced by electrical stimulation of the splanchnic nerves, and electrical recording of resultant potentials in the central nervous system. Impulses with conduction speeds of 22 to 36 meters per second were found in the ipsilateral fasciculus and nucleus

gracilis, internal arcuate fibers, and the contralateral medial lemniscus and nucleus ventralis posterolateralis of the thalamus. Impulses of 6 to 10 meters per second were found bilaterally in the region of the lateral spinothalamic tracts. Impulses of similar speed were found in the posterior hypothalamus and caudal thalamus on both sides. Impulses of intermediate speed were found in the medulla. Several techniques were employed to determine that these impulses originated from the splanchnic nerves and not from reflexly stimulated receptors. A multiple sweep recording technique permitted identification of potentials too small to be recognized in single sweep recording.

126. *Effects of prolonged administration of thiouracil on rat thyroids.* Gareth GISH and Arthur J. GATZ, Department of Anatomy, Stritch School of Medicine, Loyola University.

Littermate albino rats were used to test the effect of prolonged administration of thiouracil and 6-N propyl thiouracil on the growth of the animals and the structure of the thyroid and pituitary glands. In one group each animal received 0.025 gm of the drug daily; in the second, each animal received 0.05 gm daily. Experimental and control rats were pair fed and sacrificed 1, 2 4, 6 9 and 12 months after administration of the drug.

Daily weight records showed that the growth of the experimental rat was below normal. The thyroid follicular epithelium increased in height (about 80%) during the first 4 months of the experiment. The epithelial height gradually decreased during the 6 and 9 month periods and returned to the control level in the year period. Follicular colloid, which almost disappeared during the first 6 months, reappeared in the 9 and 12 month experimental thyroids. In the rat, the thyroid apparently becomes refractory to thiouracil administration between the 6th and 9th month and fairly normal structure appeared after one year's treatment. Hyperemia of the thyroid, which appeared during the first month, was still apparent in the year-experimental thyroids. The experimental rat pituitaries showed a decrease of alpha cells and an increase in the size and number of the beta cells during the early experimental periods.

68. *Development of the shoulder and acromioclavicular joints.* D. J. GRAY and Ernest GARDNER, Department of Anatomy, Stanford University and Wayne University College of Medicine.

The interzonal mesenchyme of the shoulder joint was denser than the surrounding mesenchyme in embryos 6 weeks of age. The long axes of the densely arranged cells were transverse to the long axis of the humerus at 7 weeks, and in some regions of the interzone a 3-layered arrangement was evident by 7½ weeks. A continuous cavity, lined by early synovial tissue, was present at 9 weeks.

A continuation of the perichondrium from the scapula to the humerus separated the interzone from surrounding mesenchyme at 7 weeks. Cellular condensations, representing a capsule, appeared at 7½ weeks and by 8 weeks collagenous fibers were scattered among the cells.

The glenoid labrum was first present at 7 weeks as a cellular condensation. It contained collagenous fibers by 8 weeks, and one week later capillaries were noted near its free margin.

Diffuse, cellular condensations for the coracohumeral, coracoclavicular, transverse scapular and coracoeromial ligaments were evident at 7 weeks. Thickenings of the capsule, representing glenohumeral ligaments, were observed at 8 weeks.

Most of the bursae around the shoulder joint were present by 8½ weeks. Others appeared shortly thereafter.

A cavity in the acromioclavicular joint made its first appearance in an 11-week fetus. This joint does not develop in a manner characteristic of the development of the larger diarthrodial joints.

90. *Kurloff bodies and lymphocytes of guinea pigs exposed to natural gas.*

Arlyne Joy GRECOL, C. Kenneth COLLINGS and John A. CAMERON, Departments of Anatomy, Baylor Research Institute and Baylor College of Dentistry.

From 15 to 20% of the leukocytes of guinea pig blood were reported by Kurloff to contain the large, brightly staining inclusions which bear his name. The twenty or more subsequent investigators of these structures have reported much lower percentages but the consensus is that they are always present in normal guinea pig blood. E. Smith from her review of the literature and her own work, concluded that Kurloff bodies are always present in "healthy, mature" guinea pigs "at a constant level."

After 1, 2, or 3 weeks of exposure to natural gas (methane) for 1 minute every 12 hours, mature females showed an average drop from 90 to 79 mononuclears and from 1 to 0.25 Kurloff bodies per 100 leucocytes. It appears that the Kurloff bodies furnish a much more sensitive index to natural gas injury than do the total mononuclears. Further studies on longer periods of exposure and on the rate and degree of recovery after exposure cease are in progress.

71. *The development and growth of the ovarian follicle of the sow.* James A.

GREEN, Department of Animal Science, University of Illinois. (Introduced by Charles W. Hooker)

The development and growth of the ovarian follicle from the primordial stage until the time of ovulation was studied in 72 normally cycling sows killed at timed intervals during the estrous cycle. The earliest primordial follicles observed were enveloped by an incomplete layer of spindle-shaped cells similar in structure to the cells of the surrounding stroma. During development these cells became more numerous, rounded up, became cuboidal and surrounded the ovum. As development proceeded several layers of follicular epithelium were formed. Mitosis was first observed in these cells just prior to the time of antrum formation. The theca made its first appearance at about the time of antrum

formation. Very little mitosis was observed in the developing theca. The theca layer consisted of spindle shaped cells until the period of estrus, at which time they became rounded up and appeared luteinized. During the 36 hours preceding ovulation the follicle wall became folded, the cells of the cumulus pulled away from the mural granulosa, and began to separate from each other.

26. *The effect of repeated matings with vasectomized males as compared with equal periods of sexual isolation on the subsequent fertility of female rats and mice.*¹ William Walter GREULICH, Cathrine CRISMON and Yoshio OKUMOTO, Department of Anatomy, Stanford University School of Medicine.

Female litter-mates were divided approximately equally between experimental and control groups, when they reached 26 to 36 days of age. Those in the experimental groups were caged continuously with potent, vasectomized males for periods of either 3 or 6 months. The controls were isolated from contact with males for the same length of time and then both groups were caged with vigorous males of proved fertility. The rats and mice used in the experiment were from our departmental animal colony and the individuals of each species were very similar genetically.

The following criteria were used in assessing the fertility of the two groups: (a) the interval between the first mating and the first litter and the intervals between subsequent litters; (b) the average number of young per litter; (c) the percentage of them alive at weaning; (d) the number of living young born to each female per 100 days of the period from her first mating until her death or her removal from the experiment; and (e) the number of these which survived till weaning.

Under the conditions of this experiment there was found no indication that coitus without reproduction, even when continued over a considerable part of the animals' reproductive life, had any deleterious effect upon their subsequent fertility. On the contrary, the findings suggest that the females which had been caged continuously with vasectomized males for periods of three to six months were subsequently more fertile than their litter-mates and other controls which had spent the same period of time in sexual isolation.

¹ Aided by grants to the senior author from the Brush Foundation and the Council of Physical Medicine of the American Medical Association.

25. *The relation between the response of the castrate male guinea pig to testosterone propionate and the precastrational sex drive.*¹ Jerome GRUNT, Department of Anatomy, University of Kansas. (Introduced by William C. Young)

In order to determine the relationship of sex behavior and certain histochemical responses to androgen, 29 male guinea pigs were castrated after having previously been tested to determine strength of sex drive. Eight additional nor-

mal animals were controls. At the time of castration the testes, epididymides, and vasa deferentia were fixed for various histochemical and histological procedures.

Following castration, the animals were tested at weekly intervals for 26 weeks. After 16 weeks, by which time drive had decreased more than 75%, 22 of the castrates were injected daily with 25 γ testosterone propionate/100 gm body weight. The remaining 7 were given sesame oil and served as castrate controls.

By the tenth week of injections the androgen-treated animals had regained 90% of their precastrational drive. At the end of the same period, the drive of the castrate controls had decreased further to less than 20% of that prior to castration.

The experimental animals responded to androgen therapy in relation to their precastrational drive. Generally, during hormonal therapy, the high-drive animals had the highest score and the low-drive animals the lowest. Since all the animals received the same amount of testosterone propionate/100 gm body weight, a somatic factor which influences the activity of male hormone is postulated.

¹ Aided by grants from the U. S. Public Health Service.

116. *Changes in the thymus, spleen, and lymph nodes of x-rayed and normal young rats resulting from the growth of heterologous tumors.* B. Vincent HALL and Katherine HAMILTON (by invitation), Division of Biological and Medical Research, Argonne National Laboratory, Chicago, and Department of Zoology, University of Illinois, Urbana.

A rapidly growing, anaplastic, mouse carcinoma transplanted subcutaneously into young rats will frequently grow vigorously for 10–14 days before regression occurs. Large heterologous tumors grow more often if the hosts are given 300 r total-body X irradiation (200 kv, hvl 0.98 mm Cu, t.d. 28½ inches, dose rate 16 r/min). Rats were sacrificed at 7, 10, 14, and 17 days, and the thymi, spleens, axillary, inguinal, and aortic lymph nodes dissected free and weighed. Histological slides and studies were made of representative sections of these organs. A significant reduction in the weight of these organs occurred after 300 r radiation. Reduction of the spleen was relatively greater than that of the thymus. The development of large, heterologous tumors induced great enlargement of the lymph nodes proximal to the tumor and significant enlargement of distal lymph nodes and spleens; but extensive reduction of the thymus. Hypertrophy of spleen and lymph nodes was most pronounced in nonirradiated animals. The combination of irradiation and a large tumor produced the smallest thymus glands. Radiation doses of 100 r had less effect on spleen and lymph node weights, little effect on thymus weight, and did not facilitate the growth of tumors. Radiation dosages of 200 r reduced the lymphoid organ weights and facilitated tumor growth much as doses of 300 r.

120. *Further observations on two types of basophil cells in the anterior pituitary.* Nicholas S. HALMI, Department of Anatomy, State University of Iowa. (Introduced by W. R. Ingram)

Two types of basophils (delta and beta cells) have been so far observed in the hypophysis of the following species: man, pig, horse, beef, rat, mouse, cat and dog. In the rat, descriptive and experimental evidence was marshalled for their separate entity (*Endocrinology*, 47: 289, 1950). Additional observations lend strong support to this concept.

In the hypophyses of two-day-old rats delta cells are abundant, especially in the females, whereas the beta cells are very sparse and have clumped filamentous material instead of granules in their cytoplasm. The acidophils are distinctly less numerous than in adults.

The pituitaries of young adult male rats treated with estradiol (50 μ g daily for 35 days) lack delta cells almost completely, while the beta cells appear unchanged. The acidophils are enlarged and degenerated.

Administration of thyroxine (0.4 mg daily for 20 days) to male rats fails to elicit conspicuous changes in the delta cells, whereas it leads to a striking diminution of the beta cell count. The acidophils are somewhat reduced in size.

The histophysiological significance and heuristic value of these findings will be briefly discussed.

29. *Patterned loss of hair on the human scalp. Types, incidence, and etiologic factors.* James B. HAMILTON, Department of Anatomy, State University Medical Center at New York City.

Eight categories of scalp hairlines were established to serve as standards for classifying and grading the extent of common baldness. These categories permit grading of 99% of scalps. Repeated examinations gave duplicate results in 99.5% of subjects.

In Caucasians scalps were studied prenatally and postnatally (62 fetuses and stillbirths; 312 males and 214 females, 20 to 94 years old; and 20 men castrated prepubertally). Seventy-seven Chinese men were also examined.

Many newborn babies become bald in areas subject to alopecia in adults. This baldness is transient and unrelated to sex.

The fullest complement of scalp hair is retained in most children until sexual maturation, and throughout life in 95% of men castrated prepubertally; it remains also in 4% of normal men and 21% of normal women. Genetic tendencies to this scalp type characterize Chinese men and some families among Caucasians.

Age-incidence curves of scalp types in Chinese men were similar in character to those in Caucasian males, but loss of hair was less extensive.

Androgens are the usual inciting agents for common baldness (Hamilton, '42), but the extent of baldness is not related to the quantity of urinary ketosteroids and axillary hair except that minimal amounts have to be present.

The interdependence of genetic, endocrine and ageing factors is analyzed.

95. *Some features of the head segmentation in Anolis embryos.* George W. D. HAMLETT, Department of Anatomy, Louisiana State University School of Medicine.

Chameleon embryos of 10 or 11 somites, with the second pharyngeal pouch just evaginating, show a pair of "head cavities" in the mesenchyme lateral to the hypophysis and posterior to the optic vesicles. These cavities represent mesodermal segment I, and in 28 or 30-somite embryos the oculomotor nerves can be traced to the walls of the cavities. No undoubted representatives of segments II or III have been found. Mesodermal segment IV is present transitorily in at least some embryos as a rudimentary somite lying posterior to the auditory placode and above the third visceral arch; it apparently disintegrates into head mesenchyme. Segments V and up form typical somites, the usually recognized first somite apparently being mesodermal segment V. The hind brain shows conspicuous neuromeres which appear in sections as a series of six transverse furrows in the floor and lateral walls of the fourth ventricle. Although these are quite constant in their relations, there is no one-to-one correspondence with the mesodermal segments; rather it appears that two neuromeres correspond to one segment, as cranial nerves V, VII and IX leave the brain opposite grooves 1, 3 and 5.

197. *Partition chromatography of free amino acids in neural tissues.* Pinckney J. HARMAN and Lloyd GUTH, Department of Anatomy, New York University College of Medicine.

Partition chromatography in two dimensions, with filter paper-water as the stationary phase and phenol-water and lutidine-alcohol-water respectively as solvent systems, permits the simultaneous visualization of all ninhydrin-reactive substances resident in living tissues. Chromatograms of tissue homogenates will detect microgram amounts of the free amino acids present in alcoholic extracts representing 50 milligrams or more of fresh tissue. In regions of cat and rat CNS which contain a combination of gray and white matter following compounds invariably occur: glutamic acid, glutamine, gamma amino butyric acid, alanine, aspartic acid, glycine, ethanolamine phosphoric ester, serine and taurine. In peripheral nerve two of these are invariably greatly reduced or below the level of detection; viz., glutamine and gamma amino butyric acid. These two substances were also absent from the cerebral cortex of a congenitally ataxic kitten and may disappear in regions adjacent to severe trauma in the CNS. Evidence is accumulating that glutamine and gamma amino butyric acid, the amide and decarboxylation derivative respectively of glutamic acid, have with the latter a special significance in the nitrogen metabolism of the neuron.

108. *Bicarbonate as an essential growth factor for chick heart fibroblasts in dialyzed media.* Morgan HARRIS, Department of Zoology, University of California.

Explants were cultured in bicarbonate-free media using Carrel flasks containing inner cups filled with NaOH to absorb CO₂. The media (plasma-serum-embryo ex-

tract) were prepared by dialysis against a balanced saline lacking bicarbonate and phosphate. Plasma and supernatants were adjusted to appropriate pH levels by adding 0.15 N NaOH just before culturing. A slight rise in pH occurs during the first few hours of incubation as CO_2 in the medium is absorbed, after which pH levels remain fairly stable for several days or more, particularly if the media are sugar-free. In the absence of bicarbonate outgrowth fails to occur at physiological levels (pH 7.2-7.8), and the margins of explants undergo disintegration. Cultures containing supplemental bicarbonate exhibit normal outgrowth at the same pH values, with or without the addition of inorganic phosphate. Increases in surface area are proportional to the bicarbonate concentration. Without supplemental bicarbonate outgrowth occurs if the pH of the medium is abnormally elevated (pH 8.0-9.5), and also if inorganic phosphate or Fischer's synthetic nutrient is added at physiological levels. In each of these cases restoration of cell proliferation parallels the accumulation of "bound" bicarbonate. The addition of Fischer's nutrient to strongly buffered dialyzed media does not increase outgrowth. These results suggest that a minimal level of bicarbonate is essential for the normal outgrowth of chick heart fibroblasts *in vitro*.

91. *The effect of cell concentration on the oxygen consumption of leukocytes under varying conditions.* John D. HARTMAN, Department of Anatomy, Western Reserve University. (Introduced by S. W. Chase)

Exudate leukocytes were obtained from normal guinea pigs: Each animal received 60 ml of a sterile 1% sodium chloride solution by intraperitoneal injection. After sixteen hours the peritoneal fluid, containing approximately 75% polymorphonuclear leukocytes and 25% mononuclear cells, was withdrawn. The cells were separated by low speed centrifugation and resuspended in buffered serum, buffered peritoneal fluid, or buffered physiological salt solution. Serial dilutions of the cells were made, each dilution being one half the cell concentration of the preceding. Oxygen uptake was measured by the direct method of Warburg and the microliters of oxygen/million cells/hour (respiration rate) plotted against the cell concentration.

That the oxygen uptake of leukocytes is not proportional to the cell concentration has been reported (Barron and Harrop, '29). For the first hour, between cell concentrations of 10,000-80,000 cells/mm³, the respiration rate of the leukocytes decreases in an inverse ratio to the cell concentration. Between concentrations of 80,000-200,000 cells/mm³ the respiration rate increases in a direct ratio to the cell concentration. With time the oxygen uptake for low cell concentrations decreases, whereas for high cell concentrations it tends to remain the same or to increase. The various experimental conditions have not affected these ratios. In any metabolic study of leukocytes the effect of cell concentration must be considered.

8. *A new staining method for the demonstration of the granules in the juxtaglomerular apparatus: the effect of nutritional deficiencies on the number of granular cells.* Phyllis Merritt HARTCROFT (by invitation) and W. Stanley HARTCROFT, Banting and Best Department of Medical Research, University of Toronto.

A new procedure has been devised whereby the granules in the afibrillar cells of the glomerular afferent arterioles are stained a deep purple. Sections fixed in Bouin's or Helly's fluid are treated with a dilute alcoholic solution of Bowie's powder and counterstained with a 0.4% alcoholic solution of Light Green (C.I. 670). Due to the intense color of the granules, a rapid, semi-quantitative assay of the numbers of the myo-endocrine cells and their granule-content is possible.

Using this technique, sections of kidneys of rats suffering from various nutritional deficiencies were studied. A striking increase in the granularity of juxtaglomerular apparatus was found in vitamin E deficiency. There was apparently a positive (inverse) correlation between the number of afibrillar cells containing increased numbers of granules and the heart weight expressed as a percent of body weight. If this finding is confirmed by experiments now in progress, it may indicate that the afibrillar cells secrete a depressor substance rather than a pressor substance.^{1, 2, 3} Indeed the known facts concerning the morphological changes of these cells may be adapted to support either the depressor- or pressor-hypothesis. It is felt that both possibilities should be kept in mind when considering the role of the juxtaglomerular apparatus in hemodynamics.

¹ Goormaghtigh, N. "La fonction endocrine des arterioles renales." Librairie R. Fonteyn Louvaine, 1944.

² Dunihue, F. W. Arch. Path., 32, 211, 1941.

³ ——— "Factors regulating blood pressure." Josiah Macy, Jr. Foundation, Transactions of the Second Conference, 1948, p. 11.
See Demonstration No. D 44.

36. *A histochemical study of Frommann's striations and a qualitative determination of chloride ions in mammalian nerve fibers.* A. HESS, Department of Anatomy, State University Medical Center at New York City. (Introduced by Gustave Noback)

There is, as yet, no direct evidence that chloride ions are present in mammalian nerve fibers. In axons, Frommann's striations can be produced by exposing to light fresh rat nerve previously treated with 1.7% silver nitrate solution containing 1.5 cc nitric acid. Since this is a reagent for chloride ions, the Frommann's striations may serve as an indicator.

In situ tests showed that Frommann's striations could no longer be revealed when nerve fibers were washed in distilled water, ammonia, sugar, potassium nitrate, or potassium cyanide solutions prior to treatment with silver nitrate-nitric acid reagent and light. Presumably, these solutions could extract chloride ions from axons. The same nerve, after extraction but subsequently immersed in sodium chloride solution, again showed Frommann's striations.

In vitro tests were performed with water extract of nerve fibers. Positive results were obtained with silver nitrate-nitric acid reagent, mercurous nitrate, and lead acetate.

Further evidence was provided by treating nerve fibers with silver nitrate-nitric acid reagent without exposure to light. The nerve was extracted with an ammonium carbonate-ammonia mixture. On neutralizing the extract with nitric acid, white silver chloride precipitated. After treatment with potassium ferrocyanide and nitric acid, the white precipitate changed color to red-brown.

It is concluded that axons of mammalian nerve fibers contain chloride ions, which react with silver nitrate to form Fromann's striations.

173. *The effects of desoxycorticosterone acetate and hypertension upon pituitary gland cytology in the rat.* Melvin HESS and C. E. HALL, Departments of Anatomy and Physiology, University of Texas Medical Branch.

Administration of desoxycorticosterone acetate (DCA) in the rat causes a reduction in the weight of the adrenal glands and in the presence of an excess of sodium chloride or ammonium chloride causes prolonged hypertension. The purpose of the present study is to determine the effects of such treatment and of the resulting hypertension on cytological structure of the anterior hypophysis.

Sixty albino rats of the Holtzman strain, half females and half castrated males, weighing between 40 and 65 grams were unilaterally nephrectomized and divided into two equal groups. A pellet of crystalline desoxycorticosterone acetate weighing 50 mg was implanted subcutaneously into each of the rats of one group and those of the other served as controls. Beginning at the 15th day of treatment blood pressure determinations were made twice weekly. At the end of the experimental period (30 days) every DCA treated rat was hypertensive (blood pressure exceeded 150 mm Hg).

Quantitative analysis of the cells of the pituitary glands revealed that a marked decrease in acidophils occurred in the DCA-treated rats, whether normal females or castrated males. The average percentage of acidophiles of the untreated group was 37.6%, whereas that of the DCA-treated group was 28.2%. No correlation was apparent between degree of hypertension and cellular structure of the hypophysis.

106. *The route of the nerves to the ovary in small animals.* R. T. HILL, Department of Anatomy, Medical Research Unit, University of Miami.

The ovarian artery, veins and surrounding mesentery have been sectioned on one or both sides of mice, rats and rabbits. Circulation is not noticeably disturbed as proven by the fact that the ovary does not blanch as from loss of blood and that in rats immediate oestrus changes are not any greater than following simple laparotomy. Tissues were collected 14 to 300 days after the cutting of the ovarian artery. The ovaries have been subjected to nerve stains and studied. Each ovary studied after section of the artery and mesentery has been found free of nerves, with control ovaries exhibiting nerves. It appears, therefore, that in these three species all nerves which reach the ovary do so in conjunction with the ovarian blood vascular system.

Data concerning ovarian function will be presented.

142. *The sequence of muscle reinnervation.* Marion HINES, Department of Anatomy, and G. Clinton KNOWLTON, Department of Physical Medicine, Emory University.

When denervation of skeletal muscle is maintained for a given period of time, each type of tissue within the muscle, denervated, passes through a definite sequence of changes which is characteristic for that particular tissue. Thus, the nerve fibers, the motor end plates, the muscle fibers, and the supporting tissues pass through several definite phases before the limit of reinnervation is reached.

167. *A study of the inorganic material which deposits in "in vitro" calcification.* Albert HIRSCHMAN, Albert E. SOBEL and I. FANKUCHEN, Department of Anatomy, State University Medical Center at New York City, Department of Biochemistry, Jewish Hospital of Brooklyn, Department of Chemistry, Polytechnic Institute of Brooklyn. (Introduced by J. P. Parnell)

The inorganic material which precipitates in *in vitro* calcification has been assumed to be normal inorganic bone salt. The present work is an attempt (1) to provide evidence on this point and (2) to study any variations in the nature of the inorganic material which deposits with variations in the composition of the calcifying solution. Immature rats were rendered rachitic to provide a zone of uncalcified cartilage. Tibias were removed immediately after sacrificing the animals. Sections 0.2–0.3 mm thick were incubated in various calcifying solutions for 24 hours at 37°C. at a pH of 7.3–7.4. These solutions had a constant calcium times phosphorus product of 45 (concn. in mg %). They varied in composition from a Ca: P weight ratio of 5: 9 to 15: 3.

In vitro calcification occurred in the epiphyseal cartilage of bones in all solutions except the controls, which contained no calcium or phosphorus. X-ray diffraction patterns from areas within the zone of new (*in vitro*) calcification and from pre-existing calcified bone showed a predominant inorganic diffraction pattern of the apatite type, characteristic of normal bone. There was no apparent difference between x-ray diffraction patterns of new and pre-existing calcification. Further x-ray diffraction and chemical studies are in progress to attempt to demonstrate more subtle differences which may still exist in the compositions of newly formed and pre-existing calcification.

10. *Adult human brain cells grown in vitro.* Mary Jane HOGUE, Department of Anatomy, School of Medicine, University of Pennsylvania.

Small pieces of adult human frontal lobe and cerebellum were grown in hanging drop and roller tube cultures using a medium of chicken plasma, chick embryo extract, human placental serum and tyrode solution. Growth of adult brain tissue was similar to that of fetal brain tissue, already reported, inasmuch as the same types of cells were observed in both. Oligodendroglia, macrophages and astrocytes appeared in the cultures early, while neurons appeared later and moved out from the explant slowly. Adult brain cultures did not exhibit the wealth of cell

processes streaming out from the explants which was a conspicuous feature of fetal cultures. Living neurons could be identified readily by the presence of long branched processes, granular cytoplasm with mitochondria and a large nucleus with one or more nucleoli. Although no neurons were observed to divide, these cells exhibited other phenomena of life and were studied as long as a week at a time.

78. *Spontaneous masculinization in old female mice of the C strain.* Charles W. HOOKER and Dorothy B. JONES, Department of Anatomy, University of North Carolina.

Approximately 50 per cent of female mice of the C strain of Strong more than 12 months of age exhibit hypertrophy of the clitoris to the extent of simulating the phallus of the male and an apparent fibrosis of the endometrium. Inasmuch as these conditions are regularly provoked in young females by administration of testosterone propionate, it may be supposed that their spontaneous development in old females is a result of the presence of endogenous androgen. The observation that the adrenal glands are alike in masculinized and non-masculinized females of the same age suggests that the androgen is not adrenal in origin. The ovaries of the masculinized females were composed primarily of old corpora lutea and nests and cords of large, eosinophilic interstitial cells, and were remarkably similar to the ovaries of mice producing androgen after many days treatment with equine gonadotrophin (Pfeiffer and Hooker, '42). Ovaries of non-masculinized females of the same age contained fewer old corpora lutea, and the interstitial cells were essentially like the usual stromal cells of the ovary.

99. *The caudal extent of the descending root of the trigeminal nerve during the period of early human fetal activity (8 to 8.5 weeks of menstrual age).* Tryphena HUMPHREY, Department of Anatomy, University of Pittsburgh.

In 3 silver impregnated human embryos of 22 mm, 25 mm, and 26.5 mm in C-R length, serially sectioned transversely, the descending fibers of V extend well into the spinal cord. The more ventrally situated ophthalmic fibers (see abstract of D 23) show relatively little difference in caudal extent during this age period, passing either to, into, or through the middle third of C2 in all 3 embryos. Obersteiner ('12) and others trace the ophthalmic division to C2 in adult man.

The maxillo-mandibular division of V not only extends appreciably farther caudalward than does the ophthalmic in all 3 embryos, but also shows a significant increase in caudal growth between 8 and 8.5 weeks, passing to C3 (left) or slightly into it (right) at 22 mm and to C4 (right) or just into it (left) at 26.5 mm. It was into C3 that van Valkenburg ('11) traced degenerated fibers of ophthalmic V in adult man.

The prominent ventral commissure in C1 and upper C2 is formed partly by secondary trigeminal fibers which, after crossing, pass through the ventral horn to join secondary ascending trigeminal fibers originating farther cephalad. Such

crossing fibers complete a reflex circuit between V and contralateral ventral horn cells which could explain the contralateral body flexion responses observed by Hooker ('39) and by Fitzgerald and Windle ('42) on stimulating maxillo-mandibular V.

180. *Lymphopenic action of epinephrine on thoracic duct lymph.* Gerald F. HUNGERFORD and William O. REINHARDT, Division of Anatomy, University of California.

To elucidate a possible mechanism for the blood lymphopenic action of epinephrine, the rate of thoracic duct lymph flow and total lymphocyte count was determined in normal and adrenalectomized 60-day old male rats which were untreated or injected subcutaneously with physiological saline solution or epinephrine in single doses of 0.07 mg per 100 gm body weight. The injection was made a half hour before lymph collection. Values are based on 10-minute samples. Rats were anaesthetized with sodium pentobarbital.

In 28 normal rats the total lymphocyte output in thoracic duct lymph was 252 ± 13 million cells per 100 gm body weight per 24 hours. The corresponding figure for 12 adrenalectomized rats was 343 ± 41 ; for 10 saline treated normal rats, 215 ± 19 ; for 15 epinephrine treated normal rats, 128 ± 10 ; and for 7 epinephrine treated, adrenalectomized rats, 302 ± 16 .

Statistically there was a significant lowering of thoracic duct lymphocytes in the epinephrine treated, normal rats, but not in the adrenalectomized rat. Since the rate of lymph flow was not affected, the chief effect was on the rate of delivery of cells to the lymph.

The conclusion is drawn that epinephrine requires the presence of the adrenal gland in order to produce a thoracic duct lymphopenia.

53. *Bladder activity of the cat in response to forebrain stimulation.* E. H. INGERSOLL, Erling S. HEGRE and Louise L. JONES, Department of Anatomy, Medical College of Virginia.

Careful analysis of the spontaneous movements of the bladder in the anesthetized cat has revealed that the various portions of the muscle rarely begin to contract simultaneously. The orderly and coordinated activity observed during the late filling stage and during micturition usually takes the form of a peristaltic wave of activity which begins first near the urethral end and passes along the long axis to the distal region of the viscus (Ingersoll and Hegre, *Anat. Rec.*, vol. 100, p. 45). Such a synchronous or peristaltic activity is a characteristic of parasympathetic responses.

It has also been shown that in response to electrical stimulation of the hypogastric nerves the detrusor exhibits a primary motor response which begins simultaneously in all parts of the bladder (Hegre and Ingersoll, *Journal of Urology*, vol. 61, p. 1043).

Recognition of these two fundamentally different patterns of activity is used to distinguish between sympathetic and parasympathetic motor responses of the bladder.

Based on these criteria an attempt has been made to elicit purely sympathetic and purely parasympathetic bladder responses by electrical stimulation of selected areas in the forebrain of the cat. Additional information is supplied by interrupting the nervous pathways involved.

135. *The morphology and vasculature of the intestinal villi.*¹ Lyle F. JACOBSON (by invitation) and Rudolf J. NOER, Departments of Anatomy and Surgery, Wayne University College of Medicine.

The intestinal villi have been studied in four species: the dog because of its frequent use as an experimental animal, the rabbit and opossum because of the similarity of their mesenteric circulation to that of man, and man as the species of chief interest. Rapid postmortem degeneration has been avoided by utilizing only specimens fixed within three minutes of interruption of their blood supply; human specimens were obtained in the operating room at the time of intestinal resection. Gross and microscopic studies have been made of freshly fixed specimens and of those which were imbedded and sectioned after injection with either India ink or red and blue latex. Observations so made have been checked by cine-photomicrography in the living animal, the findings thus recorded for repeated study by projection.

The villi of the dog are long and cylindrical, those of the rabbit roughly triangular and leaf-like, those of the opossum cylindrical with a tendency toward clubbed ends; those of man tend to be leaf-like in the upper reaches of the small intestine and more cylindrical towards the ileum. Their blood supply, derived from the sub-mucosal plexus, is through small arteries passing along the periphery of the villus towards the tip; they communicate with a central vein by capillary connections or by arteriovenous anastomoses. This situation has been further demonstrated by cine-photomicrography in the living animal.

¹This work was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

43. *Influence of niacin on liver lipids and polysaccharides.* Ralph G. JANES, Department of Anatomy, State University of Iowa.

Daily injections of 35 mg of niacin for 5 days into fasting adult female rats caused an increase in the urinary excretions of acetone bodies over that seen in saline injected controls. Liver lipids and polysaccharides were determined chemically and studied cytochemically in these rats. Total liver lipids were similar in the two groups of animals. Furthermore, when the tissue was prepared for microscopic study by osmic acid or Sudan black procedures, the lipid appeared to be similar in amount but was more evenly dispersed throughout the lobules in the niacin-injected animals. Although the chemical determination for liver glycogen were variable, increased amounts were found in many of the animals injected with niacin. This was also apparent in microscopic preparations prepared by the Hotchkiss-McManus or Bauer-Feulgen methods. The rats injected with

saline had the usual distribution of glycogen around the central vein but the rats injected with niacin had a more equal distribution of glycogen over the lobules. No definite correlation could be made in these animals between the amount of ketone body excretion and liver fat or glycogen.

157. *Efferent tracts of the lateral tegmental reticular formation in the mid-brain of the cat.* Frederic H. JOHNSON, Department of Zoology, Cornell University. (Introduced by James W. Papez)

Lesions were made with the stereotaxic instrument in the lateral tegmental formation in cats. After 14 days the brain stems were prepared by the Marchi method. The lesions interrupt fibers ascending to the following thalamic nuclei: (1) nucleus parafascicularis (3) nucleus centrum medianum, (3) nucleus arcuatus, (4) nucleus centralis medialis, and (5) nucleus paracentralis. A few fibers cross to the nucleus paracentralis of the other side. The lesions interrupted the dorsal trigeminothalamic tract coming from the main sensory nucleus in the pons. Its terminus is in the nucleus parafascicularis, nucleus centrum medianum, and nucleus arcuatus.

Lesions produced by cerebellar approach clearly show degeneration of a large tegmentotegmental decussation through the tectum. It extends forward as far as the posterior commissure. Other degenerated fibers run to the subthalamic nucleus on the same side. From a more posterior region degenerated fibers cross behind the red nucleus to scatter in the zona incerta over the subthalamic nucleus of the other side. Descending degeneration runs into the pontile reticular formation as a diffuse dorsal homolateral tract. A crossed tract passes into the nucleus reticularis tegmenti pontis. The tegmentoölivary tract is degenerated. A tegmentospinal tract courses into the spinal cord.

193. *A radiographic study of the localization of carrier-free radioberyllium in the rat.*¹ C. T. KAYLOR, Department of Anatomy, University of North Carolina.

This study was made to ascertain areas of concentration of tracer amounts of radioberyllium within organs known to be principally involved in beryllium poisoning. The available radioisotope of beryllium, Be7, is a pure gamma emitter. Gamma rays are inefficient as compared to alpha or beta particles in reducing photographic emulsions. Millicurie amounts of Be7 were injected intravenously to obtain activity levels in tissues sufficient to produce autographic images. Radioautographs of useful resolution were obtained from liver, spleen, kidney and undecalcified bone sections. Bone autographs showed intense blackening in metaphysis, marrow cavity uniformly darkened, no activity in pre-existing bone. Liver autographs showed blackening in areas corresponding to the lobular periphery. The blackening in spleen was in the red pulp.

Beryllium citrate, sulphate, chloride were injected. Distribution differed according to compound, pH and route of administration.

Tissues were cut at 5μ after alcohol fixation (which prevented leeching of Be7) and celloidin imbedding. The glass mounted sections were exposed by

direct contact with photographic emulsions. Best results were obtained with Kodak x-ray film and Ansco Process film. Intervening Au or Pt foil, 1 mil thick, between section and emulsion intensified images, sometimes with loss of definition.

¹ This work was done under contract No. At-(40-1)-266 for the United States Atomic Energy Commission.

103. *The course of visceral branches of sacral nerves to the urinary bladder in human fetuses.*¹ Donald L. KIMMEL, Department of Anatomy, Temple University School of Medicine and Elizabeth K. MOYER, Department of Anatomy, Boston University School of Medicine.

In young fetuses visceral nerves arise as 8-10 branches from the anterior rami of the 2nd-4th sacral nerves as they join the the lumbosacral trunk to form the sacral plexus. From their origin all the visceral branches course medially to lie in close relation to the inferior gluteal vessels. Most of the branches pass posterior to these vessels and continue medially to join the pelvic plexus on the lateral wall of the rectum. A few of the visceral nerves course anterior to the inferior gluteal vessels to enter the anterior portion of the pelvic plexus.

In older fetuses, due to a change in relationships, most of the sacral nerve fibers contributing to the pelvic plexus are contained in visceral nerves which originate from the anterior sacral rami proximal to the inferior gluteal vessels. In 4 of the 5 older fetuses studied additional visceral branches are present. These originate either from the sacral nerves as they enter into the formation of the sciatic and pudendal nerves, or from the pudendal nerve as it courses around the sacrospinous ligament. They reach the pelvic plexus by coursing anterior to the inferior gluteal vessels. Some follow the blood vessels supplying the bladder into the prostatic and vesical plexuses. Others course medially through the endopelvic fascia to enter the anterior part of the pelvic plexus.

¹ Aided by a grant from Hoffmann LaRoche, Inc.

57. *The reaction of sympathectomized smooth muscle to graded doses of adrenalin.*¹ Homer D. KIRGIS, Section on Neurosurgery, Ochsner Clinic and Departments of Anatomy and Surgery, Tulane University School of Medicine.

Further studies have been made in cats on the effect of adrenalin, given intravenously, on smooth muscle isolated from central sympathetic centers. The experimental muscles were the dilator pupillae and the retractor of the nictitating membrane. These muscles have been sympathectomized unilaterally by preganglionic, ganglionic and postganglionic types of operation. Doses of adrenalin (1:1000 solution) ranging from 0.05 cc per kilogram of body weight to an amount causing no reaction of the experimental muscles have been given. The minimal dose causing greater activity of the experimental than of the control muscles varies with the type of sympathectomy and the length of the postoperative period. The sympathectomized muscles whose postganglionic fibers are not intact are more sensitive to adrenalin given intravenously than those isolated by a preganglionic type of sympathectomy. The longest period of hyperactivity of the experimental muscles was 10 minutes. The degree of sensitivity has

remained essentially unchanged for as long as 48 months after superior cervical ganglionectomy. During the entire period reactions of fear and anger were not accompanied by greater activity of the experimental than of the control muscles. Following functional regeneration of the pre- or postganglionic fibers the experimental pupils were characteristically larger than the control pupils. However, upon injection of the adrenalin, there was relatively greater activity of the control than of the experimental muscles.

¹ Supported by a research grant from the National Heart Institute, U. S. Public Health Service.

30. *Relation of sex hormones to the induction and control of renal tumors in the Golden Hamster.*¹ Hadley KIRKMAN, Department of Anatomy, Stanford University.

Treatment of male hamsters, or of gonadectomized hamsters of either sex, with diethylstilbestrol continues to induce renal tumors as previously reported. Such tumors have not developed in gonadectomized or non-gonadectomized males or females receiving testosterone or progesterone together with stilbestrol. Growing, subcutaneously implanted metastases have been recovered after 100 days in stilbestrol-treated, spayed hosts, but not from non-gonadectomized, stilbestrol-treated male hosts. Regression of tumors following cessation of treatment is known to occur in non-castrates, but evidence on this point is still lacking for gonadectomized animals. Some tumor cells are now known to arise from interstitial connective tissue near the cortico-medullary border, but epithelial origins are believed to occur also. No spontaneous renal tumors of any type have been observed. There is evidence suggesting pituitary gland involvement in the induction of renal tumors. Data are based upon more than 450 animals sacrificed. Experiments are in progress designed to complete the testing of the hypothesis that in the male hamster, but not in the female, stilbestrol tends to block LH secretion as it does ACTH secretion (see Wexler, abstract 258 read by title). Since either testosterone or progesterone prevents the induction of renal tumors by stilbestrol such a sex difference in the response of the anterior pituitary gland to stilbestrol would explain the sex difference in the induction of renal tumors.

¹ This investigation was supported by grants from the American Cancer Society and the National Cancer Institute. The hormone pellets used were supplied through the courtesy of Dr. E. Oppenheimer of Ciba Pharmaceutical Products.

115. *Induction of tumors within pulmonary tissue grafts.* Arthur KIRSCHBAUM and Joyce R. SHAPIRO, Department of Anatomy, University of Minnesota.

DbA-212 mice are relatively resistant (9 percent induced tumors, 1-3 nodules), Bagg albino 100 percent susceptible (10-18 nodules) to induction of pulmonary tumors by urethane. F₁ hybrids between these 2 strains are 100 percent susceptible.

Pulmonary tissue (2 × 2 mm) of newborn DbA mice (resistant) was grafted into areolar tissue of the right ear, and lung tissue from newborn Bagg albinos

(susceptible) into the left ear of F_1 hybrid crosses. After the subcutaneous grafts had become vascularized, F_1 hybrids bearing them received 6 weekly anesthetic doses of urethane. Grafts were observed 9–13 months. Grafts were also made into F_1 hybrids not treated with urethane.

Pulmonary tumors (alveolar adenomas) appeared in 12 of 17 Bagg albino tissue grafts, whereas only one developed in 17 Dba grafts. Lungs of the F_1 hybrid hosts contained multiple urethane-induced adenomas. No tumors appeared in the grafts of hosts not injected with urethane.

Thus, pulmonary tissue of mice resistant to induction of pulmonary tumors exhibited resistance when present in hosts susceptible to induction of pulmonary tumors. Grafted tissue of intrinsic susceptibility became tumorous under identical conditions.

24. *Experiments designed to determine whether the adrenals of fetal rats are physiologically labile and whether they produce androgen.* Ralph L. KITCH-ELL, Department of Anatomy, University of Minnesota. (Introduced by L. J. Wells)

Having shown that unilateral adrenalectomy caused a compensatory hypertrophy of the remaining adrenal and that implanted steroid hormones failed to reduce the volume of the adrenal (Kitchell, '50a, '50b), there are now new observations on the series of 136 fetuses from 119 litters (72 experimental fetuses and 64 controls). The litter was the experimental unit. Treatment, begun at about 19 1/2 days after the witnessed matings and terminated just prior to expected term, lasted about 52 hours. The right adrenal and a block of the pelvic region were serially sectioned. Volume was determined from the sections and converted into mm^3 per gram of body weight (seminal vesicle and right adrenal). The left adrenal was thinly sectioned and specially stained.

Adrenocorticotrophin stimulated the growth of the adrenals, deprivation of hypophysis retarded it and castration failed to influence it. Experimentally-enlarged adrenals showed a thin outer cortical zone of small cells and a thick inner zone of enlarged vacuolated cells. With respect to the possibility that the fetal adrenal produces androgen, adrenalectomy failed to retard the growth of the seminal vesicles. Adrenocorticotrophin caused what seems to be a significant increase in the volume of the seminal vesicle ($P_t = .004$), but in castrated fetuses it failed to prevent the usual effects of castration (abstract 23). To determine whether this increase had been mediated by the fetal testes would require additional observations.

69. *The postnatal fate of the ultimobranchial body in the Syrian hamster.* Clarence E. KLAPPER, Department of Anatomy, Medical College and School of Dentistry, University of Alabama.

It has been reported previously (Klapper, '50) that only three pouches plus an ultimobranchial body are formed during the development of the pharynx of the Syrian hamster. Through the growth shifting of the pharynx and related structures, the ultimobranchial body becomes closely associated with the developing thyroid tissue approximately three days before birth.

A study was made of a series of animals varying in age from birth to about two years. The ultimobranchial body is consistently present as an irregularly shaped cyst within the central area of the lateral lobe of the thyroid. In the young individuals the cysts are lined by a simple cuboidal or stratified cuboidal epithelium. In the older animals the lining epithelium is made up of one or two layers of squamous or low cuboidal cells. The lumen of these older cysts is usually filled with a colloid which is of a different nature from that in the typical thyroid follicles. A few days after birth the cysts reach their maximum size and persist with little or no increase in volume. In adult animals many of the larger thyroid follicles are as large or larger than the ultimobranchial cysts. No definite indication that thyroid tissue is developed from the ultimobranchial body is evident. It is improbable that any significant amount of the functional thyroid has its origin from the ultimobranchial body.

83. *Knowlesi malaria in monkeys. II. A first step in separation of mechanical pathologic circulatory factors of one sludge disease from possible specific toxic factors of that disease.* M. H. KNISELY, W. K. STRATMAN-THOMAS, T. S. ELIOT and E. H. BLOCH, Division of Anatomy and Preventive Medicine, University of Tennessee Medical School; Hull Laboratory of Anatomy, University of Chicago; and Department of Anatomy, Medical College of the State of South Carolina.

During Stage II of knowlesi malaria, parasitized erythrocytes are coated with a sticky opsonizing material which permits spleen, bone marrow and liver phagocytes to ingest them selectively.

During parasite segmentation, when the parasite count rises to between 50 and 300 parasites per 100 red cells, a second precipitate forms around all blood cells, changing blood to pasty Stage III sludge. This sludge resists passage through arterioles; flow rates sharply decrease; stagnant anoxia of endothelium ensues permitting rapid plasma loss through venule walls, decreased venous return, and death.

Controls: Forty untreated monkeys died thus: all died within 12, most within 6, hours.

Experiments: High heparin dosage (two monkeys) prevented formation of opsonins and sludge, and permitted development of high parasite counts in fluid, rapidly-flowing blood. Animals with some 1000 to 1800 parasites per 1000 erythrocytes lived an additional 12 hours or more after the parasite count was above 500. Death did not occur until parasites consumed almost all hemoglobin.

Thus, Stage III sludge is lethal; it kills quicker than possible, but undemonstrated, parasite toxins.

This study permits a new approach to experimental subdivision and analysis of mechanisms of disease: 1. Recognize the lethal mechanism in an untreated disease. 2. Minimize or neutralize it, simultaneously maximizing the etiology. 3. Identify and evaluate the next lethal mechanism to appear. 4. Neutralize both lethal factors and maximize etiology. 5. Recognize next lethal factor. 6. Etc.

201. *Cytoplasmic protein in normal neurones and its morphologic and quantitative alterations in the axone reaction.* Harold KOENIG and Helmut STAHL-ECKER, (by invitation), Department of Anatomy, The Chicago Medical School.

The nucleoprotein nature of the Nissl body was demonstrated by Landström, Caspersson, and Wohlfart (1941). Hydén (1943) and Gersh and Bodian (1943), employing Caspersson's method, have studied quantitative changes in nucleic acid and protein in the axone reaction. Because their protein data is open to criticism and their studies provide little morphological information, this study was undertaken.

The Millon reaction was employed on cat spinal cord in which one sciatic nerve had been cut at varying times prior to sacrifice. Quantitative determinations of light absorption at $476\text{ }\mu\mu$ were made with a photometric technique and expressed as extinction coefficients. The cytoplasm of normal neurones is uniformly colored except for areas resembling Nissl bodies which are colored more intensely. Nuclear membrane and nucleolus give intense reactions. This reaction is prevented by peptic digestion but uninfluenced by extraction of ribonucleic acid with 10% perchloric acid.

Nerve cells undergoing the axone reaction show disruption and solution of the protein matrix of the Nissl bodies which closely resemble and parallel classical chromatolytic changes. These alterations are also seen in sections stained with the acid dyes fast-green and eosin, and the Sakaguchi test for arginine (basic proteins). Quantitative studies are in progress. Six days after nerve section neurones show a reduction in cytoplasmic protein of 7.3%, at 14 days, 32.2% and at 41 days, 17.8%.

70. *The pulmonary alveolus of the newborn mouse.* Vernon E. KRAHL, Department of Anatomy, University of Maryland Medical School.

The lungs of newborn mice and of fetal mice near term were studied as fresh preparations. Fixed and stained sections of lungs at similar stages of development were also made for purposes of comparison. The use of fresh specimens is distinctly advantageous since alveolar and capillary walls and alveolar contents may be seen clearly and are not subjected to possible alteration by the action of fixatives or other agents. The structure of the pulmonary alveolus of the mouse just prior to birth and shortly after birth is described.

204. *Gliogenous, neuronal, and indifferent cells in neuroectodermal neoplasms of the brain.* Hartwig KUHLENBECK, Department of Anatomy, Woman's Medical College of Pennsylvania.

Mixed glial and neuronal tumors of the central nervous system, although recognized by most investigators, remain controversial as regards frequency, classification, and criteria. Such tumors are variously said to constitute 0.5% (Bailey and Cushing), 2.2% (W. A. Bennett) or 12% (Globus) of the investigated series of neuroectodermal cerebral neoplasms. Willis, however, in his recent monograph

on tumors doubts that mixed glial and neuronal neoplasms occur in the central nervous system and believes that authors reporting such tumors have mistaken altered astrocytes or included nerve cells for neoplastic nerve cells.

In consideration of these problems, the features of nerve cells found in neuroectodermal brain tumors have been critically re-examined. Characteristics of neuronal elements in neoplasms are nuclear structure, chromophil substance, neurofibrillae, and cell shape. Dubious manifestation of these characteristics as well as inclusion of autochthonous nerve cells in neoplasms may indeed lead to errors in interpretation but the occurrence of mixed glial and neuronal tumors containing neoplastic true nerve cells is nevertheless emphatically upheld. There exists furthermore a neoplastic cell type which is intermediate between the spongioblastic, gliogenous, and the true neuronal type. This is believed to be a hypertrophic indifferent neuroectodermal cell.

122. *Differentiation between synthesis and release of pituitary gonadotrophin during gestation in the mouse.* A. J. LADMAN and M. N. RUNNER, Indiana University School of Medicine, Department of Anatomy, and Roscoe B. Jackson Memorial Laboratory.

Assessment of gonadotrophin content of pituitaries and responsiveness of ovaries was made in Swiss mice as gestation progressed. Both the donors of pituitary glands and the assay animals were under 6 months of age and at known stages of pregnancy. Assays were performed in a manner which would demonstrate the ovulation induction potency of one pituitary equivalent.

Responsiveness of ovaries was greatest at $17\frac{1}{2}$ days of gestation. At $8\frac{1}{2}$ days ovaries did not respond. Animals at $11\frac{1}{2}$ and $14\frac{1}{2}$ days of pregnancy responded considerably less well than those at $17\frac{1}{2}$ days. If differences in responsiveness of ovaries can be assumed to reflect secretory activity of the pituitary, then changes during pregnancy indicated release of appreciable amounts of gonadotrophin between $8\frac{1}{2}$ and $11\frac{1}{2}$ days, maintenance of the ovaries to day $14\frac{1}{2}$ and then release of additional gonadotrophin between days $14\frac{1}{2}$ and $17\frac{1}{2}$. Assay for content of pituitary glands demonstrated a drop in gonadotrophin between $6\frac{1}{2}$ and $9\frac{1}{2}$ days, a peak between $9\frac{1}{2}$ and $15\frac{1}{2}$ days and another drop between $15\frac{1}{2}$ and $18\frac{1}{2}$ days.

Correlation of ovarian responsiveness and pituitary content was interpreted in terms of release and synthesis of gonadotrophin. Observations indicate synthesis of gonadotrophin during the second quarter of gestation; concomitant synthesis and release, especially the latter, during the third quarter; and by the last quarter synthesis had dropped and/or release of gonadotrophin had increased.

141. *Regeneration of striated muscle in the rat.* Isaac LALONDE and David S. JONES, Department of Anatomy, Stritch School of Medicine of Loyola University.

The rectus abdominus muscle of the rat was transected transversely in the upper abdomen. Then the anterior rectus sheath was sutured. This procedure brought the severed ends of the muscle in close approximation. The rats were killed at 5, 10, 16, 30, 40 and 66 days. Sections were made through the healed area of the

incision in the muscle. At 5 days the area between the cut ends of the muscle fibers is filled with connective tissue. The cut ends of the transected muscle fibers are swollen, enlarged nuclei have accumulated, the striations have disappeared and buds are growing from the blunt ends. At 10 days the regenerating muscle fibers have grown through the connective tissue. The fibers are small in diameter, have large, centrally located numerous nuclei and are not striated. From this point on the fibers grow in diameter, the nuclei become smaller, less numerous and attain a peripheral position. The connective tissue becomes less and less prominent. The nerve fibers become myelinated beginning about 15 days. At 40 days the fibers are normal in appearance except for lack of a parallel arrangement. The connective tissue is reduced to a normal amount. At 66 days the new fibers seem more parallel. Microscopic and gross observations indicate that the muscle has fully recovered from the transection in about forty days.

94. *Effect of thyroid treatment on the connective tissue of Salmonidae.* G. LA ROCHE, Department of Anatomy, McGill University. (Introduced by F. McNaughton)

It was previously reported here that thyroid preparations cause an increase in the thickness of the derma of the salmon skin.

In order to test the significance of these results a series of experiments were set up, using young salmon (*Salmo salar*) of different sizes and trout yearlings (*Salvelinus fontinalis*), which had half of their beef liver diet replaced by dried beef thyroid for a period of 6 weeks. The three groups of salmon were composed of fry (3.0–5.0 cm in length), parr (6.5–13.0 cm in length) and smolt (17.0–20.5 cm in length). Corresponding groups of control fish were fed beef liver only. The treated and control trout measured 5.0–7.8 cm in length.

In all treated groups there is an increase in the thickness of the derma. This is first evident in the skin of head, opercula and fins, where the derma consists of rather loose connective tissue. The dermal hyperplasia later extends to the skin covering the body, where the derma is formed of thick bundles of collagenous fibers. In the salmon this reaction is maximal at the smolt stage and minimal at the fry stage. Trout showed an intense response extending to the connective tissue of internal organs.

146. *Development and vermian relations of the parafoveolus and the paramedian lobule.* O. LARSELL, Department of Anatomy, University of Oregon Medical School.

The dorsal parafoveolus (biventral lobule of man) is the lateral extension of the pyramis in rat, rabbit, pig and human embryos. The ventral parafoveolus (tonsilla of man) is the lateral extension of the uvula. In rabbit and pig the paramedian lobule appears before crus I and crus II are recognizable. It is delimited from the ansiform lobule by the ansoparamedian fissure. In pig embryos this becomes confluent at an early stage with a vermian sulcus, producing sulcus *a* of Bradley. In rabbits the lateral and vermian portions of the sulcus are present but they do not become confluent. A prominent vermian segment, continuous with the paramedian lobule, is formed between the pyramis and the vermian repre-

sentative of the ansiform lobule. It is designated the *posterior tuber vermis*. In rat and human embryos the paramedian lobule (lobulus gracilis of man) is late in differentiation. It is formed from the posterior portion of a crus II-paramedian lobule mass after the intercrual fissure is prominent. Medially it is continuous with a vermian subdivision corresponding to the posterior tuber, which, in the rat, is bounded rostrally by a shallow furrow that may be absent even in the adult. In the human fetus the posterior tuber and its boundaries are recognizable on the anterior wall and in the depths of the prepyramidal fissure. It continues laterally into the lobulus gracilis.

199. *Nucleolar changes in relation to development of Nissl substance of neurons.*
Arthur LaVELLE, Department of Anatomy, School of Medicine, University of Pennsylvania. (Introduced by William F. Windle)

Nerve cells of adult and fetal guinea pigs were studied with thionin and Feulgen stains. Observations were made on cells of the cerebral and cerebellar cortex, nuclei of the trigeminal and facial nerves, ventral horn of the spinal cord and spinal and trigeminal ganglia. A progressive change was noted in the staining pattern of developing nerve cell nucleoli. The homogeneously stained nucleolar bodies of the fetus became transformed to "vacuolated" adult nucleoli, the edges of which at places took up the stains much more heavily than the central region. The Feulgen reaction indicated that this change in staining pattern was due to a redistribution of desoxyribose nucleic acid in the developing nucleolus. Nucleolar changes took place concomitantly with development of Nissl substance in the cytoplasm. Various neuron groups matured at different times.

Cells in the granular layer and Golgi type II cells in the molecular layer of the adult cerebellum have embryonic type nucleoli, and show only traces of Nissl substance in their scanty cytoplasm. It would appear from this that there is an inverse ratio between the amounts of Nissl substance and of nucleolar Feulgen-positive material in the mature nerve cell.

181. *Spontaneous disappearance of hyperglycemia and glycosuria in the alloxan diabetic rat after 12-20 months of the disease.* Arnold LAZAROW and Sarah STEPHENS, Department of Anatomy, Western Reserve University.

In experiments designed to evaluate the effect of prolonged cysteine and methionine feeding on the course of alloxan diabetes, untreated diabetic rats were also studied. Weights and water intake were measured daily; the blood sugar levels were determined three times a week. Of 13 animals that survived for over one year with diabetes, seven showed a marked amelioration of their condition after 12-20 months of sustained hyperglycemia. The hyperglycemia decreased over a period of one to two months and in most cases the blood sugars returned to normal (< 150 mg/100 cc). The associated polydipsia, polyuria, and glycosuria disappeared; there was a rapid restoration of body weight toward normal.

One rat with a persistent hyperglycemia of 350 mg/100 cc (12 months) returned to a blood sugar level of 120 mg at the end of 14 months. Another rat with a persistent hyperglycemia of 250 mg/100 cc (20 months) returned to normal (120

mg/100 cc) at 22 months and it remained so for the next three months at which time death occurred. A glucose tolerance test performed at 24 months showed a decreased tolerance.

Possible explanations to be considered are pancreatic beta cell regeneration, exhaustion of the pituitary or adrenal glands, renal failure, etc. (some of these animals which have died showed obliterative glomerular lesions).

130. Influence of thiouracil on reproduction in the rat and on organ histology of offspring. James H. LEATHEM, Bureau of Biological Research and Department of Zoology, Rutgers University.

Thirty-five 30-day old female rats were fed 0.5% thiouracil in fox chow for 3 to 9 months. Mating with normal males resulted in 27 litters, 25 of which were weaned. Autopsied mothers had subnormal ovarian and adrenal weights and hypertrophied thyroids. Body weight of offspring at weaning averaged 19 grams as compared with 41 grams for controls, and thyroid weight was tripled. Normal litters nursed by thiouracil-fed foster mothers from days 3 to 22 were small and had enlarged thyroids. Only 2 of 10 litters survived more than 2 weeks after weaning time despite deletion of thiouracil. Fertile brother-sister matings were obtained with these rats.

Adrenals, thyroids, ovaries or testes, and livers were obtained from cretin offspring when 30 to 35 days old. Alkaline phosphatase, fat (Sudan IV, Sudan black B, Nile blue sulfate) Schiff, Schultz and MacManus techniques were used for histological comparison with controls. Essential differences between cretin and normal tissues were reduction of mucopolysaccharide content of thyroids, virtual absence of adrenal and ovarian fat, atrophic testes and a suggestive increase in liver alkaline phosphatase and glycogen.

140. The growth of the thyroid gland of the male rat. C. P. LEBLOND and R. PEETS, Department of Anatomy, McGill University.

Since a large proportion of the thyroid gland consists of colloid rather than cells, the question arose as to whether growth of this organ is due to colloid accumulation or to addition of cells by mitosis. This problem was approached by estimating thyroid weight, mitotic activity, diameter and number of follicles per gland, in male rats of various ages.

The weight of the gland was found to increase throughout life. Mitoses were also present at all ages. The mean diameter of the follicles gradually increased until about 2 months of age, while the number of follicles per gland remained constant at about 40,000. Afterwards, the mean diameter showed little or no increase, while the number of follicles progressively increased to reach about 90,000 in 8-10 months old animals.

It is suggested that the increase in mean follicular diameter seen before the age of 2 months is due to addition of new cells by mitosis. In older animals, many of the growing follicles reach a certain critical size, at which time they split into two or more, thus raising the number of follicles. New and old fol-

licles continue to grow in size with age as a result of mitosis. The apparent lack of increase in mean diameter after 2 months of age is due to inclusion of the small newly-formed follicles in the average.

172. *Observations on cortisone administration in normal and decapitated fetal rats.* Pierre LEROY and L. V. DOMM, Department of Anatomy and Walter G. Zoller Memorial Dental Clinic, The University of Chicago.

The stunting effect of cortisone (compound E) was first noted by Wells and Kendall (1940) on rats. Similar effects have since been observed in other animals. The present study is an attempt to determine whether cortisone has comparable effects on fetal rats.

Embryos exposed by medial abdominal incision of mother received 250–500 gamma of cortisone (Cortone, Merk) by subcutaneous injection. Results show that between 11th and 19th days of gestation a dosage of 250 gamma does not impede growth or parturition. Such embryos show normal weight at birth (average 5.8 gm). However, in 2 litters mortality was high—1 litter being reduced from 10 to 2, the other from 9 to 4 embryos. Injection of 500 gamma between 11th and 16th days killed all embryos, whereas between 16th and 19th days embryos survived and were normal in size. The same dose on 19th or 20th day resulted in loss of weight evident by 2nd or 3rd day post-partum.

Two fetuses in each of 2 pregnant mothers were decapitated on 16th day of gestation and injected with 250 gamma of cortisone. One decapitated fetus died but 3 survivors showed no perceptible growth modifications when recovered by Cesarean section on 20th day.

Since loss of weight is evident on following day when 250 gamma is administered on day after birth, it is presumed that placental circulation accounts for absence of a specific growth effect on fetus.

100. *Growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo.* Rita LEVI-MONTALCINI, Department of Zoology, Washington University.

Small pieces of mouse sarcoma 180 and 37 were transplanted to the base of limb buds of 3-day embryos. They invaded adjacent tissues, preferentially the mesonephros. From 7 days on, large nerve fiber bundles enter the tumor. The nerves are derived from sympathetic ganglia and from the later differentiating medio-dorsal cells of the spinal ganglia. Simultaneously a striking overgrowth becomes apparent in sympathetic and spinal ganglia which reaches maxima of 600% in the former and 250% in the latter. In both instances the increase in size is due primarily to a cellular hypertrophy and, to a lesser extent, to an increase in cell number.

The prevertebral sympathetic chain ganglia are also highly hypertrophic. New ganglionic complexes of visceral neurons are formed and partly incorporated in the tumor whenever the latter extends to the median plane.

In all transplants of sarcoma 37, nerve fibers from the prevertebral sympathetic ganglia invade the two mesonephroi. These organs which normally are not supplied with nerve fibers become heavily innervated by visceral fibers. The last-

mentioned effect is also obtained if the tumor is transplanted to the yolk sac and has no connection with the host embryo. We conclude that sarcoma 37 releases a substance which is responsible for the overgrowth of the prevertebral ganglia and the invasion of the two mesonephroi by their neurites.

202. *Malononitrile and nucleoprotein production in nerve cells*. Chan-Nao LIU, School of Medicine, University of Pennsylvania.

Hydén (1949) reported that administration of malononitrile was followed by a great increase in nucleoprotein production in nerve cells. In experiments similar to those of Hydén a malononitrile solution was injected intravenously into adult rabbits in dosages of 4 to 7 mg/kg. Control animals received no injections. All were sacrificed one to two hours after injection. The tissues from different parts of the central nervous system were fixed by Carnoy's or formol-sublimate solutions, or the animal was prepared by the perfusion-fixation technique using an isotonic formalin-gum acacia solution. The tissues were sectioned serially at 10 μ . For examination of nucleoproteins in nerve cells, buffered thionin and Feulgen stains were used, or photomicrography was employed with the ultra-violet light microscope at 2537 Å. Experimental and control specimens fixed by perfusion revealed no differences in respect in nucleoprotein content of neurons. Immersion fixation in Carnoy's or formol-sublimate solutions produced some darkly stained and shrunken cells scattered throughout the central nervous system in the control as well as experimental material. However, most cells were unaltered, and the changes observed appeared to be related to the nature of the fixation rather than to the administration of malononitrile.

2. *The prenatal development of the human lung*. Clayton G. LOOSLI and Edith L. POTTER, Departments of Medicine and Obstetrics and Gynecology, University of Chicago.

A histological study was made of the development of the respiratory portion of the human lung in 34 fetuses ranging in ages from 10 to 40 weeks. Fifteen were between the ages of 10 and 24 weeks. The specimens were from both live births and stillbirths and were selected for their good state of preservation. Some of the older fetuses had received artificial respiration. Several blocks from different parts of the lungs were fixed in 10 per cent formalin or Zenker formalin solution and sectioned by the paraffin technique. Some of the sections were stained with hematoxylin-eosin-azure II for histological detail, Mallory's azan stain for connective tissue and orcein stain for elastic fibers.

Our findings agree with those of Dubreuil et al., (1936) that the development of the lung may be divided into three periods (a) the glandular period up to 3½ to 4 months gestation during which the bronchial divisions are established, (b) the canalicular period (4 to 6 months gestation) during which the respiratory portion is delineated, and (c) period of formation of alveoli (6 months to term) during which definitive alveolar ducts and alveoli develop.

During the canalicular period the lung loses its glandular appearance and becomes a highly vascular organ. By following the development of the capillary

and elastic fiber systems it can be seen that the alveoli gradually develop as a result of formation of septa along the walls of the saccular channels. Thus, the alveoli are derived by the subdivision of these larger spaces which were once lined by an epithelial membrane.

136. *Some concomitants of intracorporal colchicine in vivo in mouse lung, spleen, liver and other tissues.* Charles C. MACKLIN, Department of Histological Research, the University of Western Ontario.

Six hours after intraperitoneal injection of 0.1 mgm colchicine in 0.25 ccm distilled water, the lungs of well grown albino mice fixed by one day's immersion of the fresh skinned unopened thoraces in Regaud's or Bouin's fluid show the capillaries of the alveolar walls packed with white corpuscles of which many resemble hemoblasts. Clusters of chromosome-like bodies not seldom appear in the latter in place of nuclei. Such formations, which seem to originate by an atypical mitotic process, are also occasionally seen in alveolar wall epithelial cells. These lungs thus contain "mitotic" figures in immigrant cells, presumably dislodged from lymphoid and marrow tissue by colchicine action, as well as in lung cells. Similar "chromosome" complexes are found in cells in metacapillary blood, and in spleen. Polychromatic erythrocytes and erythroblasts are abundant. Hordes of fine dark-brown granules in the plasma and many large similarly colored bodies in the alveolar epithelial cells appear after fixation with strong formalin. The livers show great dilation of the bile ducts and the small intestines are swollen with presumably hepatogenous fluid. Diarrhea is marked. The consequent dehydration of the blood may well favor lodgment of free cells in the lung capillaries.

119. *Genetic aspects of human breast cancer.* Madge T. MACKLIN, Departments of Medicine and Zoology, Ohio State University.

The genetic aspects of human breast cancer have been studied. Women are selected from two populations; (1) those related to breast cancer patients; (2) control cases related to non-cancer patients of the same age as the breast cancer cases. They are matched in pairs for all of the following items; being alive; being dead; decade in which death occurred; age; type of relationship to the original patient interviewed. Sisters are matched with sisters, mothers with mothers, etc. The members of the pair are then compared for the presence or absence of breast cancer. Only the pairs in which the members are dissimilar in these respects are usable in the study. All pairs whose members are alike in either having breast cancer, or not having breast cancer, are discarded. Usable pairs are plotted as points on a graph between two parallel lines, whose slope and distance apart are determined by formulas the values of which are set by the investigator. When the plotted points cross the upper line, the idea is accepted that women related to breast cancer patients have breast cancer in significantly higher numbers than have women from the control population. Heredity probably plays a leading role in this similarity. Such acceptance has been reached for a number of relationships such as sisters, paternal aunts, etc.

7. *An analysis of semen quality in fertile and infertile men — 2000 cases.* John MacLEOD, Department of Anatomy, Cornell University Medical College.

The various aspects of semen quality (sperm counts, motility and morphology) are analysed statistically in 1000 men of known fertility and in 1000 men from cases of infertile marriage. It is shown that the previous standards for the normal range of male fertility so far as sperm counts are concerned are much too high. The essential difference between the fertile and "infertile" males lies at the "under 20 million/cc" sperm count level. Above that level, there is no increase in potential fertility. In similar fashion, the qualities of sperm motility and morphology have been analysed in the two groups and the differences will be discussed.

21. *Anatomical studies of the human anterior tibial muscle.* J. E. MARKEE, Department of Anatomy, Duke University School of Medicine.

The anterior tibial muscle has multiple origins, insertions, functions, and innervation and is especially subject to paralysis. This report deals particularly with its multiple innervation.

The nerves to the tibialis anterior are usually five in number. The *first nerve* arises from the recurrent articular, and enters the deep posterior lateral border of the tibialis anterior near its origin and innervates the upper lateral muscle fibers. The *second nerve* arises from the recurrent articular, enters the deep surface of the tibialis anterior between the muscle belly and the tibia near the muscle origin and supplies the anterior and medial fibers of the upper half of the muscle. The *third nerve* arises from the recurrent articular branch in common with the first nerve to the extensor hallucis longus. It enters the deep posterior-lateral border and supplies the upper middle third of the muscle. The *fourth nerve*, a branch of the deep peroneal, enters the deep posterior-lateral border of the muscle almost on the interosseus membrane, and innervates the superficial surface of the muscle belly, and the central and posterior lateral fibers of the middle third of the muscle. The *fifth nerve* arises from the deep peroneal nerve, and enters the posterior-lateral border of the tibialis anterior in its lower third. It divides into two branches which give off several short filaments each but the main fiber continues to the tendon.

In the dog these separately innervated areas can be made to contract separately.

176. *Maintenance of life and of body weight by daily administration of either adrenal cortex extract or ACTH in rats with bilateral hypothalamic lesions.* Leo C. MASSOPUST, Jr., Clifford SHERWOOD, Dorsey E. HOLTKAMP, Robert M. HILL and A. R. BUCHANAN, Departments of Anatomy and Biochemistry, University of Colorado School of Medicine.

(Abstract withdrawn.)

152. *The topography of the nucleus ambiguus of the cat.* Howard A. MATZKE, Department of Anatomy, State University Medical Center at New York City. (Introduced by A. T. Rasmussen)

This investigation was carried out in order to furnish more accurate information as to size, extent, position and localization of the nucleus ambiguus. The ninth, tenth and eleventh cranial nerves were sectioned individually or in combination in the cranial cavity or neck in cats. All operations were performed on the right side. The animals were allowed to survive for varying periods of time, then killed and perfused. The brains were removed, sectioned and stained with cresyl violet or thionin. The nucleus was outlined by noting the chromatolysis in serial sections of the medulla. The optimum time for chromatolysis varied from 7 to 25 days depending on the level at which the nerves were sectioned.

The nucleus ambiguus contains approximately 1050 cells unilaterally. It is 3.5 mm long, extending from the junction of the pons and medulla to a point just below the obex. The nucleus is largest and most distinct in its cephalic portion where it is continuous with the nucleus of the facial nerve. Inferiorly its cells blend in with those of the eleventh nucleus. Although there is some overlap, the cephalic portion is concerned with the innervation of the pharyngeal musculature by way of the glossopharyngeal and pharyngeal branch of the vagus. The inferior portion of the nucleus sends its axons via the vagus to the muscles of the larynx.

64. *Alkaline glycerophosphatase in the developing thyroid, pituitary, and parathyroid of the albino rat.* Richard J. McALPINE, Department of Anatomy, Indiana University. (Introduced by John H. Van Dyke)

The changes in localization of alkaline glycerophosphatase in the developing thyroid, pituitary, and parathyroid of the albino rat were studied by the histochemical method. In the case of the thyroid, the period of differentiation is accompanied by a marked phosphatase activity in the cell cords derived both from the median thyroid anlage and from the ultimobranchial tissue. Adult thyroid cells are characterized by only slight nuclear activity. The decrease in phosphatase activity occurs prior to colloid formation. Cystic remnants of ultimobranchial tissue in postnatal thyroids exhibit marked phosphatase activity.

In the case of the pituitary, the activity of the pars neuralis decreases gradually during embryonic life from the intense activity seen in its earliest stages. The pars anterior and pars intermedia exhibit an increase in activity above that seen in the primitive Rathke pouch epithelium, followed by a marked decrease in late fetal life. By contrast, the pars tuberalis exhibits a direct and early decrease in activity from that of Rathke's pouch epithelium. The parathyroid gland, in contrast to thyroid and pituitary, exhibits no such increase in phosphatase activity during its development.

These results give further evidence of the multiplicity of chemical differentiative pathways which exist, depending both on the source of the precursor cells and upon the particular definitive cellular function to be produced.

42. *Cytochemical studies on the testes of rats.* William B. McENERY and Warren O. NELSON, Department of Anatomy, State University of Iowa.

Testes from normal, hypophysectomized, cryptorchid, and estrogen treated rats were examined by the following procedures: Sudan black, Nile blue, Schiff plasmal, birefringence and Schultz.

Normal animals. All of the reactions are positive for intertubular areas while tubules give all reactions except birefringence and Schultz in the distribution described previously (McEnery and Nelson, 1950).

Hypophysectomized animals. All reactions are negative for intertubular areas. The tubules give a positive reaction for all tests. The droplets are chiefly large and located principally in the basal region of the tubules. This constitutes a marked deviation from the normal in that there is an increase in amount of lipid and absence of the four distinct patterns present in the normal (McEnery and Nelson, 1950).

Cryptorchid animals. The intertubular areas give a positive reaction for all tests. The tubules present the same picture as in hypophysectomized animals; however, the number of tubules showing a reaction is not always uniform. This appears to be related to the presence or absence of germ cells, in the latter case the reactions being negative.

Estrogen-treated animals. The intertubular areas in this group are positive for all procedures. With the exception of the Schultz reaction the tubules are positive for all tests, and the distribution corresponds to that in hypophysectomy. It must be noted, however, that the Schiff reaction and birefringence are not a constant feature of the tubules.

165. *Minor variations of the vertebrae of the mouse associated with the short-ear gene.* Wallace McNUTT, Department of Anatomy, University of Texas Medical Branch.

The vertebrae of three strains of mice were observed for intra- and interstrain differences. Each of these strains of mice has been bred brother $\times$ sister with forced heterozygosis for the short-ear gene, in excess of the generations required for each to approach homozygosity for all genes except this one, and those very closely linked with it. In all three strains the transverse process is either reduced in size or absent altogether in selected portions of the vertebral column in mice homozygous for short ear. Perhaps associated with this is a deficiency in the cervical vertebrae causing the transverse foramen to be imperfect laterally (costal element absent in *se se* mice). This shows a preference for certain cervical vertebrae and is expressed a little differently in the three strains. The first and third cervical most often escape the defect. The anterior tubercle of the sixth cervical vertebra is absent in short-eared mice in different frequencies in the three strains. Abnormalities observed in the atlas and axis, presence or absence of the neural spine on the second thoracic vertebra, and spina bifida showed no significant association with short ears in these data. While vertebrae of short-eared mice weigh less than those of their normal-eared brothers the differences seem to be selective in effect, rather than just a uniform reduction in size.

105. *An analysis of the lumbar sympathetic trunk in the dog.*¹ William R. MEHLER, Department of Anatomy, Saint Louis University. (Introduced by William F. Alexander)

The myelinated fiber components of the sympathetic trunk in the dog have been analyzed from the levels of T12 through S1 after the following operative procedures: (a) spinal nerve sections just distal to the spinal ganglion from T13 through S3; (b) ventral root section from T13 through S3; (c) spinal ganglion extirpation from T13 through S3. After time lapse for complete degeneration, the sympathetic trunks were removed, stained with osmic acid and sectioned serially. After spinal nerve section most of the myelinated fibers in the trunk were degenerated. However, a significant number of medium and small myelinated fibers were still present in the middle and lower levels of the trunk. These fibers are probably afferents from higher levels. No myelinated fibers were found in communicating rami in the lower levels after this procedure. Following ventral root section the medium sized myelinated fibers were found to be absent. In the trunks studied after spinal ganglion extirpation, all the large and the majority of the medium to small myelinated fibers were found to be degenerated.

¹ This work was supported in part by grant H-14, Division of Research Grants and Fellowships, U.S.P.H.S.

37. *Histochemical study of developing adipose tissue in guinea-pigs under the influence of vitamin E.*¹ Z. MENSCHIK, Department of Anatomy, School of Medicine, University of Ottawa.

Histochemical study of interscapular, axillary, inguinal and subperitoneal adipose tissue was made in fetuses, and in young and adult guinea-pigs. The latter were fed five different diets. The total number of animals investigated was 114.

Two kinds of adipose tissue were encountered; white and brown. Histochemical studies showed that brown fat in comparison with the white is richer in mucoproteins, alpha-amino acid groups, water-soluble polysaccharides, free fatty acids, unsaturated fatty acids, phospholipids, glucolipids, cholesterol, amine oxidase, alpha-naphthol oxidase and cytochrome oxidase.

The adipose tissue of brown fat character appears first during development in the second half of fetal life. It is usually encountered first in the interscapular region.

Vitamin E-deficiency promotes the development of brown adipose tissue and inhibits the development of white fat. This is more conspicuous when sesame oil is added to vitamin E-deficient diets.

Vitamin E seems to be necessary for the formation of white adipose tissue.

In adult animals vitamin E-deficiency produces the gradual disappearance of white fat; however, the brown adipose tissue is preserved.

¹ Supported by a grant from the National Research Council, Canada.

20. *Blood supply of pancreas.* Nicholas A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Vascularization patterns are markedly different and coextensive with variations of celiac axis and its branches. Dissection of 200 specimens revealed following typical arterial arrangement

(1) Two pancreaticoduodenal arcades about head of pancreas, an anterior formed by the superior pancreaticoduodenal, a posterior formed by the retroduodenal (first branch of gastroduodenal). Arcades unite with superior mesenteric via a separate or common inferior pancreaticoduodenal. Arcades may be double, triple, entirely or in part.

(2) Dorsal pancreatic, regularly distributed to back of neck region but of varied origin (splenic, 39%; celiac, 22%; superior mesenteric, 14%; hepatic or right hepatic, 12%; aberrant right hepatic, 7%; middle or left colic, 4%; gastroduodenal or right gastroepiploic, 2%). Typically, it has two right branches (one joining superior pancreaticoduodenal, the other supplying uncinate process) and a left branch, the transverse pancreatic. A fourth branch frequently descends below pancreas to communicate with the superior mesenteric, middle colic or accessory middle colic and in instances (4 mm) is actually the middle colic.

(3) Transverse pancreatic which courses along inferior surface of pancreas at tail end of which it anastomoses with a. pancreatica magna and a. caudae pancreatis . . . branches of splenic or its terminals. Often arises from gastroduodenal, superior pancreaticoduodenal, inferior pancreaticoduodenal, superior mesenteric.

(4) Pancreatic rami (2-10) from splenic trunk. (5) Rami from hepatic, gastroduodenal, right gastroepiploic. (6) Rami from right gastric and supraduodenal of Wilkie, the two often forming a suprapancreatic arcade with branches to pancreas.

49. *Cutaneous localization following 180° rotation of skin grafts.* Nancy MINER, Department of Anatomy, University of Chicago. (Introduced by R. W. Sperry)

Recent studies have indicated that the integument induces a refined local specificity in sensory neurons during development of cutaneous local sign. Further evidence of this integumental specification of cutaneous fibers is presented here.

On 15 tadpoles a rectangular strip of skin 1 cm wide, extending from near the mid-dorsal to near the mid-ventral lines, was excised, rotated 180°, and replaced so that the dorsal skin was located ventrally and vice versa. As expected, the grafts underwent self-differentiation with development of their typical pigmentation patterns despite the atypical orientation.

After metamorphosis, localizing responses elicited by tactile stimulation of the rotated graft were aimed erroneously in correlation with the intrinsic specificity of the graft rather than the topography of the body surface. Touching the white ventral skin on the back, innervated by dorsal rami of spinal nerves, elicited reflex rubbing of the belly by the forelimb. Tactile stimulation of the dorsally pigmented skin on the belly, innervated by ventral rami, elicited reactions of

the hind limb aimed dorsally at the original site of origin. Stimulation of the lateral skin in its normal position elicited typical low wiping responses of the hind limb aimed at the lateral body surface. Stimulation of skin surrounding the rotated implant produced normal localization.

The results support the conclusion that sensory fibers are induced to undergo a local chemical specification as a result of their peripheral contacts with the integument and that this specification in turn helps to regulate the formation of central reflex relations in accordance with the chemoaffinity theory of synaptic formation.

110. *The effects of tyrosine on the pigmentation of embryonic chick skin cultured on synthetic media.* Floyd E. MORBECK and John W. Saunders, Jr., Department of Biology, Marquette University. (Introduced by W. Zeit)

Isolates of skin (ca. 2 mm by 5 mm) from the back and saddle areas of 6½–7½ day Barred Rock embryos, cultured for 24 hours at 37°–38°C. on a basal medium of buffered salt solution (Ringer-Locke), agar (450 mg/%), and glucose (850 to 1000 mg/%), show an essentially normal differentiation of melanophores (cells bearing granules of melanin pigment), but fail to form feather germs.

Tyrosine, possibly the substratum for melanin synthesis, was added to the basal medium (16 mg/%) to ascertain its effects on the differentiation of melanoblasts (prospective pigment cells). In 34 out of 44 cases, explants cultured in the presence of added tyrosine showed more numerous expanded melanophores with darker pigment granules than did control explants, but were otherwise similar in appearance.

Addition to the basal medium of phenylalanine (16 mg/%), an amino acid differing from tyrosine only in its lack of an —OH group, produces no apparent effect on the pigmentation of similar explants.

These results suggest that tyrosine in the culture medium acts directly to accelerate melanogenesis, possibly by providing an increased concentration of substratum available for melanin synthesis.

Currently this possibility is being tested in experiments designed to determine the effects of varying the concentration of tyrosine in the medium on the rate of pigment synthesis in skin isolates.

46. *The differentiation of connective tissue from argyrophilic fibers within the kidney of the cat.* Russell E. MORRIS, Department of Anatomy, Emory University. (Introduced by Marion Hines)

In order to differentiate nerve fibers from connective tissue, the enzymatic digestion techniques of Mall were used. Following digestion of kidney tissue with pepsin and trypsin, the argyrophilic fibers of the renal glomerulus were found unchanged and presented the characteristics of reticular connective tissue. Following denervation of the kidney by extirpation of the sympathetic trunk and coeliac ganglion, by section of the splanchnic nerves, and by stripping of the

renal pedicle, no alteration of the reticulum was found. The nerve fibers in the region of the glomerulus do not penetrate it. Rather they are restricted to the blood vessels. This nerve plexus can be differentiated from the surrounding reticulum.

159. *Assessment of physiological age by a combination of several criteria: vision, hearing, blood pressure and muscle force.* Irwin M. MURRAY, Department of Anatomy, Dalhousie University and State University Medical Center at New York City. (Introduced by James B. Hamilton)

A method is presented for assessing physiological age in an individual based on the examination of five physiological functions in a sample of 38 business and professional men between the ages of 21 and 84 years. The five physiological functions tested were (1) range of visual accommodation, (2) sensitivity of the dark-adapted eye, (3) auditory acuity, (4) systolic blood pressure and (5) strength of hand grip. Age is expressed in terms of a combination of the five physiological functions and the squares of those functions. The distribution of the deviations between the estimated physiological ages and the chronological ages of the 38 men indicates that the equation is equally applicable at all age periods. The standard error of the estimated physiological age was ± 7.35 years. The method can be used with an unlimited number of physiological, anatomical and psychological criteria. The usual methods of expressing age in terms of physiological function do not enable one to combine the various functions to give a single index of physiological age. So far as is known no previous attempt has been made to combine two or more physiological functions for an assessment of physiological age.

3. *The distribution of fat in the spleen after intravenous injections of fatty chyle and fat emulsions.* Raymond G. MURRAY, Department of Experimental Medicine, Northwestern University.

Olive oil given to rats by stomach tube could not be detected in frozen sections of the spleen stained for fat. When artificial emulsions of olive oil were mixed with rat blood in vivo or in vitro, the fat immediately agglomerated into large droplets. In vivo these droplets accumulated rapidly in the marginal zone of the red pulp, mostly outside the phagocytes. The fat in the marginal zone later appeared in the macrophages and moved into the central red pulp. By 24 hours, in most cases, the spleen was free of fat. Fatty dog chyle similarly introduced in rats remained finely dispersed and no fat accumulated in the marginal zone.

Similar results were obtained with dogs although the particles of artificial emulsion did not agglomerate so rapidly or completely as in rat blood. The one marked difference was the rapid and striking concentration of both chyle fat and artificial emulsion in the ellipsoids. No ellipsoids, or fat accumulations suggestive thereof, were found in rats.

These observations have been interpreted as supporting the views that (a) there are no ellipsoids in the rat spleen, (b) in the dog, blood flows out between the reticular cells of the ellipsoids into the pulp spaces, and (c) the circulation in the spleen under some circumstances is of the "open" type.

137. *Structure of liver in testosterone anaesthesia.* Paul Foley NACE, Department of Anatomy, The New York Medical College and Flower-Fifth Avenue Hospitals. (Introduced by E. Lawrence House)

Fifty female Vermont *Rana pipiens* were studied after treatment with testosterone and other agents. Without preliminary treatment and after treatment with liver poisons, animals received anaesthetic doses of testosterone by intraperitoneal injection. Behavior and response to stimuli were observed. After varied periods, up to 24 hours, livers were removed and fixed in Bouin's fluid and Heidenhain's Susa fluid. Sections were stained with modified Masson trichrome stain.

Hepatic parenchyma cells of testosterone anaesthetized animals were found free of basophilic cytoplasmic granules which form a prominent feature of untreated controls and of animals poisoned with several other substances. The bile canaliculi of testosterone treated animals appeared significantly enlarged. Other changes observed less consistently.

189. *Quantitative estimation of radioautographic reactions of thyroid follicles.* N. J. NADLER and R. BOGOROCH, Department of Anatomy, McGill University. (Introduced by L.-F. Bélanger)

The amount of radioiodine present per follicle at various time intervals after injection of radioiodine was estimated by counts of photographic grains in radioautographs of thyroid glands.

Male rats were sacrificed at various time intervals after radioiodine injection and "coated radioautographs" of their thyroids were prepared. The radioautographic reaction produced by each follicle was measured by counting grains at definite levels in a $75 \mu^2$ area over the center of the colloid.

It as shown mathematically that the same concentration of radioactivity in follicles of various sizes would result in a different number of grains in the photographic emulsion. Theoretical correction factors were, therefore, calculated; and their accuracy was tested by counting grains of autographic reactions produced by radioiodinated albumen strips of various widths.

Using proper corrections, the true concentration of radioactivity was found to be greater in smaller than in larger follicles soon after injection. Later, this concentration decreased rapidly in the smaller follicles, so that after 200 hours they contained less than the larger follicles. This result suggests a faster turnover of radioiodine material (e.g., thyroglobulin) in the smaller follicles.

150. *Interrelationships between cortex and thalamus in spindle-bursts.* William T. NIEMER, Department of Anatomy, The Creighton University School of Medicine.

In cats under "Dial" anesthesia, bipolar EEG recordings were taken simultaneously from strychninized cortical areas and thalamic nuclei. Periodically occurring, waxing and waning runs of 8 - 10/s waves commonly called thalamic bursts or spindles were studied in relation to thalamic distribution and interrelationship with the cortex.

Spindles ranging in amplitude from 200 - 700 microvolts were recorded from all the thalamic nuclei but none could be obtained from the lateral geniculate body. They occur most frequently in the region of the ventromedial nucleus.

Of large functional thalamic groups the intrinsic relay nuclei showed the greatest tendency to spindle while the associational, diffuse associational and extrinsic relay nuclei, in the order given, spindled to a lesser degree.

Strychnine spikes originating in the cortex can repeatedly trigger such burst activity in the thalamus. Such triggering occurs mainly from the sensorimotor and associational cortices but may also be obtained from the auditory and limbic regions. Further study may reveal more specific corticothalamic interrelationship in the spindle function.

200. *Histochemical changes in the myelin sheath during Wallerian degeneration.*¹ Charles R. NOBACK, Department of Anatomy, Columbia University.

The myelin sheath (sciatic nerve of rat) is colored by Sudan black, is tinted faintly by Sudan IV, is stained by Baker's phospholipid technique, is dyed blue but not red by Nile Blue sulphate and is anisotropic. The sheath possesses cholesterol, free aldehydes (Schiff reaction), plasmals and periodic acid-Schiff positive lipids.

For approximately 10 days after the transection of a nerve, the myelin sheath products show the same histochemical properties as the myelin sheath of an untransected nerve. These observations indicate that during this period, the fragmentation of the myelin sheath is not accompanied by observable histochemical changes in the sheath lipids.

From 10 to 40 days following transection several histochemical changes are noted. Sudan IV intensely colors the granules and some droplets that are present throughout this period. After 20 to 25 days, myelin products rarely reveal free aldehydes or plasmals, while several ovoids are stained red by Nile Blue sulphate during the limited period, 20 to 30 days after transection. The myelin sheath products (except for granules and some droplets) reveal the presence of cholesterol or its esters, periodic acid-Schiff positive lipids, anisotropic lipids and lipids yielding positive reactions with the Baker technique. These histochemical data are indicative of chemical changes occurring during this period. All myelin sheath products of Wallerian degeneration are usually colored by Sudan black.

Aspects of these observations will be discussed.

¹ Aided by a grant from the Coordinating Council for Cerebral Palsy in New York City.

143. *On the presence of nerve tissue in the media of the human ductus arteriosus.*
Gustave J. NOBACK, F. David ANDERSON and W. Gregory COOPER,
Departments of Anatomy, University of Puerto Rico School of Medicine
and Cornell University Medical College.

This study is concerned primarily with a detailed histological analysis of the human ductus arteriosus, pulmonary artery, and aorta in relation to the physiological closure and anatomical obliteration of the human ductus arteriosus. The specimens were removed at autopsy and included pulmonary artery, ductus arteriosus, and aorta. These specimens were fixed, sectioned and mounted serially, and alternate sections were stained with, 1. hematoxylin and eosin, 2. Weigert's elastic tissue stain, 3. Masson trichrome stain, and 4. Modifications of Bodian's Protargol nerve stain.

The intima of the ductus in our material is characterized by the presence of areas of "loose tissues" arranged in pads of button-like encroachments into the lumen, but these are not as marked or as extensive as usually described in the literature. The inner elastic membrane is continuous from the pulmonary artery through the ductus and into the aorta. The "loose tissue" is also present in the media of the ductus to a greater extent than that in the intima in the same general location. This "loose tissue" is suggestive of mesenchyme and possibly represents a de-differentiation.

Examination of our material definitely shows the existence of nerve bundles, individual nerve fibers with branchings, sub-terminal nets, and terminal arborizations suggesting presso-receptor structures in the media of the human ductus arteriosus.

The presence, as here demonstrated, of nervous tissue in the media of the ductus arteriosus must be considered in attempts to understand the mechanisms involved in the closure of this vessel.

63. *The comparative growth rates of developing wings and legs of the chick embryo.*¹ Wiktor W. NOWINSKI and Wasley D. YUSHOK (by invitation),
Department of Neuropsychiatry, University of Texas Medical Branch.

It has been often suggested, but never clearly demonstrated, that the wings and legs of a developing chick embryo grow at the same rate up to the 5th or 6th day and from then on the wings grow slower and the legs faster. In our experiment on the enzymatic pattern of the developing limbs of the chick embryos, it became essential to establish the growth rate on a quantitative basis. The multiplicative growth rate was measured by the content of desoxyribosenucleic acid (DNA), estimated by the modified Schneider method. All analyses were done on several samples of pooled material. Stages from the 6th until the 12th day were investigated. The curves of DNA showed very similar values at the 6th day (0.017 mg DNA per wing against 0.019 mg per leg); however, in the course of further development, the curves showed progressive differences (e.g., 0.03 mg DNA/1 wing and 0.04 mg/1 leg on the 7th day; 0.05 mg/1 wing and 0.10 mg/1 leg on the

8th day, and finally reached 0.29 mg/1 wing and 0.65 mg/1 leg on the 12th day). These curves of growth rates serve as a fundamental basis for the identification of the enzyme systems which are primarily involved in the ontogenesis of the fore and hind limbs.

¹This work was supported in part by grant No. RG 2167, Division of Research Grants and Fellowships, U.S.P.H.S.

47. *Correction for use of stereotaxic instrument for monkey skulls of different sizes.* Jerzy OLSZEWSKI, Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute.

During the preparation of an atlas of the thalamus of the monkey (see demonstration), it was observed that the frontal plane of a given structure varies from one animal to another. In older animals with longer heads, the position of the external acoustic meatus is more caudal; hence, a given anatomical structure has a higher frontal plane numeral in those animals. However, data obtained on the basis of the external measurements have an approximate value only; more precise information may be obtained from x-ray studies. The position of the external auditory meati, and preferably that of the inferior orbital ridge also, should be marked by small metallic pieces, and then the lateral view x-rays are made. The best measurement on which the correction of the frontal plane may be based is that of the distance between the anterior clinoid processes and the frontal plane O. This distance equals 18 mm in the animal used for preparation of the photomicrographs for the atlas. In other investigated animals this distance varied from 18 to 26 mm. In order to apply the frontal plane as given in the atlas to another animal, this animal should be x-rayed and the "anterior clinoid-O plane" distance should be measured. Then the difference between the obtained numeral and 18 should be added to the numeral of the frontal plane in the atlas.

164. *Congenital carpal anomalies.* Ronan O'RAHILLY, Department of Anatomy, Wayne University. (Introduced by Gordon H. Scott)

Congenital anomalies of the human carpus have been examined in a series of several hundred routine skiagrams. Apart from minor variations in shape, size, or position of the individual bones, the anomalies may be classified as: (1) Absences, reductions, mass fusions, or duplications associated with antebrachial or metacarpal abnormalities; (2) Isolated fusions between two or three carpals—some of the factors influencing joint cavitation and differentiation in the embryo are discussed in relation to the development of "fusions"; (3) Accessory ossicles—the embryological basis of accessory elements has been investigated in serial sections, and comparisons are made with the findings of Trolle (1948) on the foot; (4) Bipartition, seen most frequently in the scaphoid, and considered by some to be always traumatic in origin. The possible modes of formation are discussed.

98. *A study of hydrocephalic brains of prenatal and postnatal rats from dams on a diet deficient in vitamin B₁₂.*¹ M. D. OVERHOLSER, J. R. WHITLEY, B. L. O'DELL and A. G. HOGAN, Departments of Anatomy and Agricultural Chemistry, University of Missouri.

The hydrocephalic rats were obtained from dams fed an experimental diet deficient in vitamin B₁₂ (Hogan, O'Dell, and Whitley, '50). Over 20% of the newborn are hydrocephalic.

Serial sections of 7 normal and 25 hydrocephalic brains (1 to 90 days of age) were studied. The latter showed markedly distended lateral and third ventricles and cerebral aqueducts which were abnormal in contour in the anterior portion; two showed complete closure in the midportion of the cerebral aqueduct. The fourth ventricle appeared normal in all cases.

Two females, which had produced at least three consecutive litters containing a high percentage of hydrocephalic young, were sacrificed on the 16th and 18th day of gestation respectively. Embryos of the same age were taken from normal females as controls. The six 16-day embryos from the experimental female showed no abnormality in any part of the ventricular system. The cerebral aqueduct was completely closed in its mid-portion in six of the nine 18-day embryos and partially closed in three. The lateral and third ventricles were distended while the fourth ventricles appeared normal in all cases.

These findings indicate that the hydrocephalus is produced by a closure of the cerebral aqueduct in the embryo (by the 18th day of gestation), and that the aqueduct is opened later, presumably by the increasing pressure of the cerebrospinal fluid.

¹ Aided by a grant from the U. S. Public Health Service.

112. *Doubts raised by tissue culture studies of the importance of the bone (alkaline) phosphatase in osteogenesis.* George H. PAFF and Bennett SALLMAN, Divisions of Anatomy and Bacteriology, Hahnemann Medical College.

In 1947 we reported that more calcification occurs if pH is lowered. The reverse should be true if bone phosphatase is essential. Recently we found ('50) that alizarin could selectively block osteogenesis. Since then, attempts to identify this block cast further doubts on the importance of phosphatase in osteogenesis. It is not caused by phosphatase inhibition. Assayed phosphatase solutions lose no activity in vitro, following addition of alizarin. No significant differences were detected in the visualization of phosphatase at the site of bone formation.

Apparently the block is not due to a calcium lake formed by alizarin. Calcium ions are available to the extent that there is no interference with clotting of plasma. Again, 50-hour embryonic chick hearts function normally in the presence of alizarin. This is impossible without calcium ions.

Nor do other changes in the media cause the block. If osteogenesis is stopped by alizarin, it will not begin again when the bone is transferred to a dye-free medium (Paff and Eksterowicz, '50). We have repeated this experiment being

careful to wash the bones before placing them on dye-free medium. The same negative result was obtained.

These observations are admittedly not conclusive, but if they do nothing other than concentrate more attention on the "local factor" they will be useful.

62. *Phosphorus fractions in the chick embryo.* Mary Hanson PARTRIDGE and Charles K. WRIGHT, Department of Anatomy, Emory University School of Medicine. (Introduced by Josel Szepsenwol)

Phosphorus fractions were determined in various tissues of chick embryos throughout development, using the procedures of Pinchot, Bloom and Close.

In membrane, total phosphorus increases from the 10th to the 15th day, decreasing gradually thereafter. The variation is due chiefly to changes in the inorganic and hexose phosphate fractions; the other fractions show only minor changes throughout development.

Total phosphorus of the heart increases between the 9th and 11th days, then again increases slightly on the 19th day. Inorganic phosphate more than doubles its concentration between the 10th and 14th days, subsequently increasing slightly with minor variations. Hexose phosphate, after an initial increase between the 9th and 12th days, shows no variation except a slight rise just before hatching. Phosphocreatine and ATP fluctuate slightly, an increase in one being accompanied by a decrease in the other.

Intestinal smooth muscle shows little variation in total phosphorus. The increase in the inorganic fraction noted between the 11th and 18th days is compensated by a decrease in the organic fractions.

The most striking changes are observed in skeletal muscle. Total phosphorus increases slightly up to the 12th day, then rises abruptly until the 19th day, decreasing slightly before hatching. The increase is chiefly in inorganic phosphate, and partly in hexose phosphate. An increase in ATP is accompanied by a decrease in phosphocreatine, and vice versa.

67. *The possible embryological mechanisms involved in the genesis of exstrophy of the bladder and epispadias.* Bradley M. PATTEN, Department of Anatomy, University of Michigan.

The clue to the embryological mechanisms involved in the genesis of exstrophy of the bladder lies in the correct interpretation of the commonly coexisting epispadias. Hypothetical stages in disturbed development are postulated which seem to explain the manner in which these associated anomalies may arise. The starting point is with embryos young enough to show the genital tubercle primordia still in their primary paired condition. The initial departure from normal is believed to be the formation of these primordia too far caudally with reference to the proctodaeum, so they are located at the level at which the urorectal fold will present externally. The corpora cavernosa would then develop just caudal to the urogenital outlet, and the urethral groove would form in their dorsal angle instead of its usual location in their ventral angle. This would establish the relations characteristic of epispadias. The same abnormal position of the genital tubercle would entail, also, an absence of the

ingrowth of mesenchyme which normally converges toward the mid-ventral line just cephalic to the urogenital orifice. Thus when the cloacal membrane ruptures there is no mesodermal bar to its cephalic extension. The likelihood of its breaking through all the way to the umbilicus, as is characteristic of exstrophy, is enhanced by the exceedingly rapid elongation occurring at this time in the region between the urogenital orifice and the umbilicus.

58. *Some effects of oxidized vitamin C on the central nervous system.* John W. PATTERSON, Department of Anatomy, Western Reserve University.

The intravenous injection in rats of dehydroascorbic acid, the reversibly oxidized form of vitamin C, produces a marked reaction which is characterized by extreme hyperactivity, salivation, and lachrymation. The hyperactivity can be prevented by anesthetic doses of nembutal or urethane. At the time that hyperactivity would normally occur there is a rise in the blood pressure of the anesthetized animal. This rise is blocked by dibenamine and by lumbar sympathectomy with splanchnicectomy. The hypertensive response is observed in decapitated animals when larger doses are administered. These facts indicate that the response is the result of a central stimulation of the sympathetic nervous system. Lachrymation and salivation are prevented by atropine; and salivation is prevented by blocking the chorda tympani nerve. These facts indicate that these responses are the result of a central stimulation of the parasympathetic nervous system. Following the injection of dehydroascorbic acid, but not ascorbic acid, the level of ascorbic acid in the brain may be doubled or tripled within a few minutes. This reduction within the nerve cell of a neutral substance, dehydroascorbic acid, to an acid substance, ascorbic acid, probably releases acetylcholine from its inactive precursor with a resultant excitation of the central nervous system. This reaction may be of value in the treatment of certain psychological disorders.

155. *Observations on the posterior gray columns of the spinal cord.*¹ Anthony A. PEARSON, Department of Anatomy, University of Oregon Medical School.

This study is based primarily on Golgi preparations of spinal cords of newborn cats and human (infants). The gelatinous substance is composed mainly of small multipolar neurons whose processes are limited to that region. There are larger multipolar neurons scattered in the posterior, lateral, and medial margins of the gelatinous substance. Their coarse freely branching dendrites are also limited to the region of the gelatinous substance. The cells just ventral to the gelatinous substance are medium sized multipolar neurons. Their dorsally directed dendrites are restricted to a rather narrow zone of the gelatinous substance. The deeper cells are often a little larger and the spread of their dorsal dendrites may include the dendritic zone of several of their dorsal neighbors. The ventrally directed dendrites are lost in the adjacent gray matter. The axons of the above cells could not be followed for any great distance.

Numerous fibers leave the posterior funiculus and Lissauer's tract and enter the posterior gray columns. Many of the coarser fibers by-pass the gelatinous substance by passing on its medial and lateral sides. Some go directly through it to reach the deeper cells of the posterior gray columns. Many of the smaller fibers terminate in the gelatinous substance. The pattern of the branching and distribution of these fibers will be described.

¹ Aided by a grant from the U. S. Public Health Service.

125. *Permeability of the placenta of the thyroidectomized female guinea pig to thyrotrophin.*¹ Roy R. PETERSON, Department of Anatomy, University of Kansas. (Introduced by Henry C. Tracy)

Twenty animals were thyroidectomized. After a 30-day recovery period 8 were found in heat and mated. Beginning at the 38th day of pregnancy, they were injected subcutaneously with 5 Junkmann-Schoeller units (5 mg) of thyrotrophin (Parke-Davis) daily.

Four females resorbed the embryos between the 20th and 40th day of pregnancy, 1 aborted on the 54th day, and 3 delivered normal litters.

To check on the transplacental passage of TSH, determinations of oxygen consumption, heart rate, thyroid and pituitary weights were made on the 5 living offspring delivered by the 3 TSH-treated females, and on 8 normal newborn animals. Data were obtained as follows: oxygen consumption, controls, 199 cc/100 gm/hr (174 to 228), experimentals, 166 (105 to 238); heart rate, 300/min. (246 to 342) and 321 (282 to 360), respectively; relative thyroid weights, controls, 27.3 mg/100 gm (21.7 to 32.5), experimentals, 27.1 (23.1 to 29.8); relative pituitary weights, 5.2 mg/100 gm (4.9 to 5.8), and 5.0 (4.0 to 6.7), respectively.

These data indicate that excessive quantities of TSH may have some deleterious effect on pregnancy in thyroidectomized guinea pigs but not the drastic effect reported by Glaubach (1934), who treated intact pregnant females. The placenta does not appear to be permeable to thyrotrophin, since the thyroids of the offspring were not enlarged.

¹ Aided by grants from the U. S. Public Health Service.

28. *Hormonal control of growth and pigmentation of the nipple in mice.* Carroll A. PFEIFFER, Department of Anatomy, Yale University.

Androgen is extremely effective in producing growth and pigmentation of the nipple in mice. Local application of 2.0 μ g of testosterone will produce grossly visible pigmentation of the nipple within 4 days in ovariectomized mice of the C₅₇ strain and maximum enlargement and pigmentation within 6 weeks. Estradiol benzoate (in doses under .2 μ g per day locally) causes enlargement, but only occasionally pigmentation, of the nipple. Larger doses give both enlargement and blackening. Progesterone applied locally in doses of 2 mg per day apparently produces neither enlargement nor pigment formation. In combination with estrogen it does not enhance the estrogen response and even may be slightly inhibitory. Progesterone also delays the first appearance

of pigmentation and may possibly slightly inhibit the enlargement of the nipple by testosterone. In the dosages used, estrogen plus testosterone shows either no effect or a slight additive effect over that of testosterone alone. Intact female mice respond equally well. Nipples are absent in male mice, and no skin pigmentation was elicited by application of testosterone to that area in the mammary line. Nipple enlargement can be produced in albino mice, but no pigmentation occurs. The nipples enlarge during pregnancy, but in the C₅₇ black strain little if any pigmentation develops even when the pregnant mouse has had several previous litters and is still suckling young.

59. *Grafting experiments between pigmentless and normal eggs of Amblystoma punctatum.* Jean PIATT, Department of Anatomy, School of Medicine, University of Pennsylvania.

In *Amblystoma punctatum* melanin granules are present within the cells of the central nervous system and ganglia. Ectodermal derivatives, in general, possess more of the pigment but it is found in many other tissues as well. A single clutch of pure white, pigmentless eggs was obtained last spring. The larvae and adults subsequently developed a normal pigment pattern and chromatophores were distributed throughout the body in characteristic fashion. Melanin pigment was completely missing, however, in all cells other than chromatophores. The single observed exception to this was the retinal tapetum. Reciprocal grafting experiments were performed between these white eggs and the normally pigmented embryos of this species. The intracellular melanin granules served as natural markers in tracing the origin and migration of various cells and cell groups.

Observations were recorded on: (1) The derivatives of transplanted cranial and trunk neural crest material, i.e., neural fold. (2) The origin of some of the cranial ganglia. (3) The migration of the caudal portion of the VIIth motor nucleus. (4) The extent of contralateral migration of trunk neural crest.

101. *Sympathetic rami in the frog.* Joseph PICK and Donal SHEEHAN, Department of Anatomy, New York University College of Medicine.

Recent physiological studies on vasomotor and secretory responses in the sympathetomized frog have emphasized the need for a fuller knowledge of the anatomy of the autonomic nerves in amphibia. A series of dissections have therefore been made of the sympathetic trunk in the frog (*Rana pipiens*) in order to record the common variations occurring in sympathetic ganglia and rami communicantes. These investigations were supplemented by histological studies of the sympathetic rami and spinal nerve roots at all levels, using osmic acid and silver techniques. A distinction between grey and white rami communicantes is not possible. The sympathetic rami are all composed of unmyelinated fibers interspersed with fine myelinated fibers at 2-4 μ in diameter. The limitation of the sympathetic outflow from the 2nd to the 7th ventral spinal roots and of the spinal parasympathetic outflow from the 9th to the 10th nerves as proposed by Langley and Orbeli could not be confirmed by these preparations. Fine myelinated fibers are present in small numbers in all ven-

tral spinal roots including the 1st and 8th nerves. In the frog therefore there does not appear to be the same clear demarcation anatomically of sympathetic and parasympathetic spinal outflows as found in higher animals.

111. *Effects of some biologically active compounds on skeletal muscle in vitro.*¹

Irene A. POGOGUEFF and Margaret R. MURRAY, Department of Surgery, College of Physicians and Surgeons.

Skeletal muscle in tissue culture furnishes a valuable test object for muscle-active drugs, since such preparations are entirely devoid of nervous connections, including end-plates, and contain intact, isolated fibers which often engage in spontaneous rhythmical contractions.

In the experiments reported here, a system of quantitative evaluation for contraction has been introduced, whereby each culture is rated before, during and after an experiment, on a scale ranging from 0-16. The average rating of a series of cultures may thus be obtained, and the stimulating or depressing effects of experimental agents may be expressed numerically.

Earlier tests with purified ryanodine, and extremely potent curari-like alkaloid (Merck), produced precise and predictable results: a paralysis whose latent period and duration were closely correlated with concentration in the medium. It was not clear, however, whether the contractile substance of the muscle was influenced, or whether contraction was blocked at the cell membrane. For comparison and contrast, four other biologically active compounds have been tested on similar cultured muscle material: procaine and veratrine (regarded as membrane active), caffeine (in doubt) and mapharsen (regarded as affecting the contractile proteins). When this series of results is compared with the data of other workers on non-conducting fiber preparations, it is concluded that ryanodine probably acts at the membrane, rather than directly on the contractile proteins.

¹ This work was done under a research grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

102. *An analysis of the nerves along blood vessels in skin and striated muscle of the hindlimb of the cat.* Edward H. POLLEY, Department of Anatomy, St. Louis University, School of Medicine. (Introduced by Albert Kuntz)

In the central footpad and the muscles of the hindlimb of the cat the autonomic nerves to the blood vessels were examined after the fibers interrupted by sectioning the spinal nerve roots from the 2nd lumbar to the 2nd sacral segments had undergone degeneration. The sensory nerves were studied after degeneration of the sympathetic nerves following extirpation of the sympathetic trunk from the level of the 2nd lumbar to the 2nd sacral segments. The operations were carried out unilaterally and the opposite side was used as a control. Holmes' silver nitrate technique was used most extensively. Some sections were stained by Bodian's Protargol, and some nerves were stained with osmic acid.

The nerve supply to the subepidermal vascular plexus is mainly sensory. Sympathectomy causes no appreciable change in the perivascular nerve plexuses, but section of the spinal nerve roots results in degeneration of nearly all the fibers

in these plexuses. In muscle after spinal nerve section, a few large myelinated nerve fibers, probably sensory fibers descending through the intact sympathetic trunk from levels higher than the 2nd lumbar segment, remain intact. Large vessels exhibit a plexus in the adventitia and the outer layers of the media which include both sensory and sympathetic fibers. Smaller vessels exhibit a less abundant nerve supply which is made up more largely of fine fibers, possibly sympathetic. Sympathectomy does not cause an appreciable reduction of the number of fibers in the outer adventitia, but the fibers at the junction of the adventitia and the media are reduced in number.

160. *Innervation of the uterus.* Hugo C. PRIBOR, Department of Anatomy, St. Louis University School of Medicine. (Introduced by Norman M. Sulkin)

The extrinsic innervation of the uterus has been studied by gross dissections, and the intrinsic innervation by the use of silver stains on serial sections of newborn human and cat uteri. The extrinsic nerves are derived through the pelvic plexuses. The sympathetic supply is derived via the hypogastric plexus, and the parasympathetic supply via rami from the second and the third and often the fourth sacral nerves.

Large nerve trunks enter the uterus at the cervix along blood vessels. Their branches extend to all parts of the body and the fundus. The musculature and the blood vessels in the myometrium are abundantly innervated. Some nerves extend into the endometrium. They are most numerous in its basal third. Some fibers pass within a few cell layers of the epithelium but none have been observed to enter the epithelium. The cervix is more abundantly innervated than the rest of the uterus. No ganglion cells have been observed within the wall of the uterus. An attempt is made to estimate the relative abundance of sympathetic, parasympathetic and afferent nerve fibers in the uterus.

139. *Observations on bile conducting surfaces in mammals.* Paul H. RALPH, Department of Anatomy, University of Washington.

The surfaces of liver cells, bile capillaries, bile ducts, hepatic ducts, gall bladders, cystic ducts, common bile ducts and points of junction of the common bile duct epithelium with the epithelium of the crypts of Lieberkühn in the duodenum have been examined in rat, monkey, and guinea pig. Certain details of morphology, not previously reported, are revealed if thin sections ($1\mu \pm$) without stain are mounted in media of selected refractive indices and examined with the Dark M phase microscope. The walls of bile capillaries show evidence of spiral internal surface sculpturings. The occurrence of a granule at the points where the two juxtaposed grooves in the surface of adjacent liver cells meet to form the capillary is not uniform. Terminal bars, which at certain refractive indices ($1.390-1.402 \pm$) appear as tiny open canals, are displayed by all epithelium-lined portions of the bile conducting system. Filamentous surface structures (striated border) are displayed by interlobular bile ducts, hepatic ducts, gall bladders, cystic ducts, and common bile ducts, and in some forms by the larger intralobular ducts. Their size and number vary in different species. In no case are they as large or as

numerous as in the intestine. Negative images of the Golgi apparatus located between the nucleus and the lumen are easily visible in most cells exhibiting filamentous structures at their surface. Changes in the bile conducting system following some experimental procedures are described.

114. *Growth characteristics of transplanted liver parenchyma (mouse)*. A. J. RAMSAY, The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The liver primordia of mouse embryos, and liver from mouse fetuses, newborn, suckling, weaned, and adult mice were transplanted to the ears of suckling and newly weaned mouse hosts. Liver taken from adult mice 3 and 6 days after partial hepatectomy was transplanted, also.

The results in general were negative. Transplanted liver primordia from 10½ day embryos grew only for a few days, then degenerated. In only 18% of this series could liver cells still be seen at 3 weeks. Cysts of well differentiated stomach and intestine, normal pancreatic material, and cystic bile duct formations were commonly produced. Transplants of liver from older embryos, fetuses, and postnatal animals grew very little if at all, although differentiation was rapid. Vascularization of the transplants was unsatisfactory; as a rule, and was the only observable explanation for the poor growth. Liver transplanted several days after partial hepatectomy of the donor grew slightly better than the others and its survival was of similar duration.

It is concluded that liver will not grow as well as most other tissues when transplanted to the ear, vascularization of the implant is unsatisfactory, and its survival is limited. It is felt that liver parenchyma requires peculiar vascular relationships for proper growth, differentiation, and survival. Series of experiments wherein transplanted liver parenchyma has access to greater vascularity, and to portal (vein) blood, are under way.

85. *Further evidence for intracorpuseular para-crystallization of hemoglobin in sickle cells*. J. W. REBUCK, R. M. STURROCK, R. W. MONTGOMERY and E. A. MONAGHAN. The Henry Ford Hospital.

The sickling process was followed in electron micrographs of erythrocytes obtained from sickle-cell trait and anemia patients. Cells at differing anoxic stages were prepared by a modified Beck-Hertz technic; some were shadow-cast with chromium.

Elsewhere we have presented electron micrographs of the sickling process depicting early anisotropoid angulation of central hemoglobin aggregates and later intracorpuseular massing of hemoglobin with intense peripheral spiculation as evidence of incipient crystallization.

At this time electron micrographs of sickle cells are shown in the configuration of tactoids (spindle and other shapes) and in configurations transitional between tactoids and the classical sickled forms. These findings tend to confirm the observations of Harris made with the light microscope. In addition shadow-cast sickle-cells are presented as evidence for the tridimensional structure of the hemoglobin aggregates and their peripheral spicules. The projections of varying

heights are evidence of spicule rigidity. In thinner areas, orientation of what appear to be hemoglobin-containing aggregates into elongated fibers or rods can be observed. The largest aggregates visible at relatively low ($\times 6000$) magnifications are not necessarily parallel; this observation helps to account for the diversity of outlines of fully sickled cells. The smaller constituents of the linear, intracorpuseular aggregates may well be parallel. Obviously, orientation of hemoglobin-containing aggregates has been compromised by the forces inherent within the surface ultrastructure. Whether or not the surface ultrastructure is defective remains to be determined.

104. *The origins of the splanchnic nerves.*¹ Adrian F. REED, Department of Anatomy, Tulane University School of Medicine.

The origins of the splanchnic nerves have been determined in dissections of 100 cadavers. The sources of the greater splanchnic nerves varied widely, 58 patterns being found. The most common arrangement occurred in 13 nerves (6.5%). This type consisted of greater splanchnic nerves formed by rami from the 6th, 8th and 9th thoracic sympathetic ganglia. Only 2 bodies had bilaterally symmetrical patterns. The highest ramus to the greater splanchnic nerve came from the 4th thoracic ganglion in 4 cases, the 5th ganglion in 64, the 6th ganglion in 85, the 7th ganglion in 41, and the 8th ganglion in 6.

The range of origins of the lesser splanchnic nerves was from the 9th to the 12th thoracic ganglia. The most common type (27.5%) was in the form of 2 rami, one originating from the 10th ganglion and one from the 11th.

The least splanchnic nerve arose by varying numbers of rami from either the 11th or 12th ganglia or from both. Fifty-seven percent were formed by one ramus from the 12th ganglion. The least splanchnic nerve was absent bilaterally in 2 bodies and on 1 side in 3.

In this series of dissections, in addition to the splanchnic nerves 570 very small nerves, mainly to the aortic and some to the celiac plexuses, were present. They originated from the sympathetic chains, between the 6th thoracic and 1st lumbar ganglia.

¹ Aided by a grant from the National Heart Institute, U. S. Public Health Service.

60. *Lens regeneration in Salamandra salamandra salamandra.*¹ Randall W. REYER, Department of Anatomy, University of Pittsburgh and Leon S. STONE, Department of Anatomy and Osborn Zoological Laboratory, Yale University.

Extensive investigations have shown that lens regeneration from the dorsal iris margin occurs in all species of the genus *Triturus* which have been studied (13). Early experiments of Fischel and co-workers indicated that this phenomenon might also occur in *Salamandra salamandra*, but more slowly. It seemed desirable to repeat the experiments on this species since negative results had been obtained in a reinvestigation of some other urodeles and anurans, previously reported as regenerating a lens.

The lens was removed unilaterally or bilaterally in 144 larvae of *Salamandra salamandra salamandra*. These were killed at frequent intervals up to 50 days following the operation. Lens regeneration, similar to that in *Triturus*, occurred

but from the temporo-dorsal rather than mid-dorsal iris and at a slower rate. A depigmented vesicle appeared after 12 to 18 days, primary fiber pole after 20 to 24 days, secondary lens fibers after 24 to 26 days and detachment from the dorsal iris after approximately 37 days. Early regeneration stages differed from those in *Triturus* larvae in the more gradual depigmentation of the cells at the iris margin, quite frequent asymmetry in lens fiber formation and retention of some melanin granules within the regenerated lens.

These results show that Wolffian lens regeneration will occur in at least these two genera of the family Salamandridae.

¹ Aided by grants from the U. S. Public Health Service and The James Hudson Brown Memorial Fund of the Yale University School of Medicine.

86. *The reaction of peripheral blood leucocytes "in vitro" to certain carcinogen crystals.*¹ Kenneth M. RICHTER, Department of Histology and Embryology, University of Oklahoma School of Medicine.

Controlled "in vitro" studies employing micromanipulative, phase and polarizing microscope techniques were made on the reaction of leucocytes of *Rana pipiens*, *Crotaphytus collaris* and of man (O, A, B, AB blood types and one case of myeloblastic leukemia) to microcrystals of methylcholanthrene, benzpyrene and dibenzanthracene. Neutrophils and eosinophiles in human and their homologues in frog and lizard blood aggregate on and phagocytize crystalline methylcholanthrene, benzpyrene and dibenzanthracene. In experimental preparations, mitochondria, golgi complex, specific granules, chromatin etc. of all leucocytic types showed no morphologic alterations from normal, irrespective of the amount or whether the crystals were intracellular, extracellular or both. Neutrophils and eosinophiles only, in both control and experimental preparations, produced and discharged cytoplasmic vacuoles. Carcinogen crystals, regardless of their cellular relationships, had no apparent effect on the motility or longevity of the various leucocytic types; in both control and experimental preparations the leucocytes after 4-5 days rounded up and died. Crystals contained within living cells for 72 hours showed no evidence of being digested nor did cell death subsequent to this time effect any physical change in them. Crystals, whether intracellular, extracellular or both, did not alter the vital staining reaction of the leucocytes to neutral red or janus green.

¹ Supported by a Grant-in-Aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

39. *Alkaline phosphatase activity of healing experimental wounds of gingival and cutaneous epithelium.* J. R. RING, Department of Anatomy, Washington University School of Dentistry.

Epithelium of normal gingiva and skin of the rat contains relatively small amounts of active alkaline phosphatase enzyme as determined by histochemical methods. Slight enzymatic activity is evidenced only in keratohyaline granules and in the nuclei of the more basally placed cells.

Gomori preparations of these membranes 24-72 hours after the infliction of short incisions indicate marked increase in activity of the enzyme in the vicinity of the healing wound. Increased cobalt sulfide precipitation is observed in all strata of the epithelium, but it is most intense in the keratinizing layers. With completion of epithelial healing, phosphatase returns to the low level of the normal tissue.

117. *Tumors of guinea pigs.* James Boyles ROGERS, Department of Anatomy, University of Louisville School of Medicine.

The incidence of tumors in guinea pigs of an inbred colony over a 10 year period is reported. The colony consists of families which are the descendants of a female ancestor. The animals have been fed oats, cabbage, alfalfa hay, salt and water. The animal room temperature has been 72°F. from October to May, and has varied with the general atmospheric temperature in the summer.

Approximately 150 animals have survived more than 1095 days. Of these, 50 are still alive. Post mortem examination has been made on about 600 animals of both sexes and all ages. Twelve tumors have been found at post mortem and one tumor has been diagnosed clinically in a living animal. Ten of these tumors were found among 100 senile animals at post mortem. Two were found in animals which survived less than three years. Five tumors were found in individuals of one family, and two tumors in each of three other families. The tumors have been classified as: ovarian cyst, lymphoblastoma, lipoma and fibroma. Age and familial inheritance are factors in the incidence of tumors in guinea pigs.

144. *Resorption and deposition of osseous tissue.*¹ Elbert B. RUTH, Department of Anatomy, University of North Dakota.

Studies on resorption and deposition of osseous tissue were made on the middle thirds of the diaphyses of female rats maintained on a calcium-free diet while suckling large litters. Under these conditions the amount and approximate rate of resorption and/or deposition is known from previous studies. Resorption is first noted (in the compacta) by the appearance of irregular chromophobic streaks in the interstitial substance. As these streaks widen they show specific staining reactions, according to the stains used. Fissures appear in these areas, widen, and become spaces of various shapes and sizes. Narrow rims of unaffected interstitial substance remain about each osteocyte and its lacuna in the area, for a time. These too are dissolved away finally, leaving empty spaces in the compacta. Notable is the absence of cells of any kind in these spaces while they are forming. Cells invade the space subsequently—osteoblasts, macrophages, reticulum cells, fibroblasts, occasional osteoclasts, with collagenous fibers and reticulum fibers. Resorption is not due to activity of osteoclasts. It is the result of action of an osteolytic substance in the plasma that functions when a protective substance in the tissue itself is neutralized or

absent. Deposition is effected through interaction of a secretion of the osteoblasts with osteoplastic substances in the plasma to form the calcified ossein that is osseous tissue.

¹ This work was supported by a grant-in-aid from the American Cancer Society.

148. *Projection of "vestibular" cerebellar areas to the cerebral cortex.* M. M. RUWALDT and Ray S. SNIDER, Department of Anatomy, Northwestern University Medical School.

Cerebellar folia within the nodule, uvula and pyramis were electrically stimulated in the cat and the surface of cerebral hemispheres explored for evoked electrical responses.

The major responses were located in two cerebral regions: (A) an area approximately 10 mm in diameter overlapping the anterior ectosylvian and anterior suprasylvian gyri, and (B) anterior and posterior sigmoid gyri. Region A is coextensive with that to which Walzl and Mountcastle found a vestibular nerve projection and overlaps Woolsey's somatic II area. Region B is located in the sensorimotor area. Generally, region B is more responsive during pyramis stimulation while region A is more responsive during uvular and nodular stimulation, although there is considerable overlap. Responses of approximately 4, 7 and 15 milliseconds latency were observed. Projection pathways are being investigated as are other "vestibular" areas of the cerebellum.

107. *On the choice of media in diagnostic tissue culture.* Machteld E. SANO,¹ Department of Pathology, Women's Medical College and Hospital of Pennsylvania.

For the last ten years pathological tissue removed in the operating room has been studied in tissue culture using autogenous, homogenous and heterogenous plasma whenever possible. Clinical studies with follow-up correlated with histopathology and tissue cultures of 100 lymph nodes, 70 brain tumors and 60 pleural effusions clearly demonstrate that for diagnostic purposes, autogenous and homogenous plasma is preferable to heterogenous plasma though there are occasional exceptions. These observations listed and correlated on the lantern slide show that in the advent of a good synthetic medium, autogenous, homogenous and heterogenous plasma will still have to be used concomitantly if tissue culture as an aid to clinical and tumor diagnosis is to be evaluated in the years to come.

¹ Aided in part by grant from the Committee on Growth and from The A. Barr Chase Cancer Research Foundation, Temple University Medical School and Hospital where studies were pursued.

22. *Gallbladder variations in the domestic rabbit.* Paul B. SAWIN, Roscoe B. Jackson Memorial Laboratory.

A gallbladder is not necessarily a species characteristic of the domestic rabbit. More than 10% of the individuals in one family line of New Zealand Whites and approximately 6% in a randomly selected hybrid population of 1275 in-

dividuals lack this organ. According to size, the gallbladders of these populations and of 2205 individuals from 25 other races (some of them highly inbred) can be classified in continuous series with approximately regular frequency distributions, but varying in their range. Some races have small gallbladders including a complete lack of this organ as one extreme type, and others have large bladders including some as large as 103.1% of the length of the liver lobe. Racial bladder size is not correlated with body size. Evidence obtained from three sources—from comparison of racial differences in old and newborn bladders; from comparison of racial differences in bladder size with the known regional enlarged growth areas of the same races; and from study of variations in shape—all point to genetic differences in growth rate of the regional type as the cause of these variations.

74. "*Spontaneous*" ovulation in the estrogen-treated rabbit and its seasonal variation. Charles H. SAWYER, Department of Anatomy, Duke University School of Medicine.

The rabbit ordinarily ovulates only after neurogenic stimulation of the adeno-hypophysis at coitus, and this stimulus is effective in activating release of ovulating hormone (LH) at any season. Although estrogen facilitates LH release in the rabbit in response to nervous and chemical stimulation, earlier attempts to induce "spontaneous" ovulation with estrogen led to negative results. Following combined estrogen-progesterone treatment, however, a few rabbits did ovulate without other extrinsic stimulation. The present work reports that prolonged treatment with estrogen alone induces spontaneous ovulation during certain seasons.

Mature female rabbits were treated on 4 consecutive mornings with subcutaneous doses of 85 μ g estradiol benzoate in peanut oil, and the ovaries were examined at laparotomy 48 hours after the last injection. During April this treatment resulted in ovulation in 5/11 animals. In May only 1/17 responded, and during June, August and October, 0/32 ovulated. During December, 2/16 ovulated and in February, 3/11.

Thus the ovulatory mechanism is more sensitive to estrogen during winter and spring than in summer and fall. For various reasons the site of estrogen action is considered to be at the nervous or the hypophyseal level. The seasonal variations may be correlated with seasonal changes in sensitivity of the LH-release mechanism to artificial stimuli and in pituitary gonadotrophin content.

156. *On detailed connections of the medullary and pontine reticular formation.* Arnold B. SCHEIBEL, Department of Psychiatry, University of Illinois School of Medicine. (Introduced by Ray S. Snider)

Recent electrophysiological studies of the brain stem reticular formation indicate the necessity for reevaluation of the detailed anatomy of this structure. Modified Golgi silver impregnation techniques were used to study both medial and lateral cell groups in new-born mammals. Control series of Nissl stained preparations were used.

Medial cell groups including the anterior magnocellular formation of Cajal contained cells ranging in size from 35-80 micra and possessed dendritic ramifications approaching 1/3 of the total cross section of the brain stem. Generally, the very large ventral-lying cells sent dendritic processes to ipsilateral and contralateral pyramidal tracts. Dendritic processes were also followed to M.L.F., medial lemniscus nuclei of the sixth nerve and tractus solitarius.

Dendritic processes of lateral cell groups were observed in the area of the ipsilateral nuclei of trapezoid body motor seventh and inferior olive. More lateral processes passed to nucleus and tract of the descending trigeminus.

Axones were more difficult to follow and additional studies are being made to establish their cephalad and caudad extent.

121. *Effects of amino acid deficiencies on the gonads and anterior pituitary.*¹

E. B. SCOTT, Department of Anatomy, C. SCHWARTZ, Department of Biochemistry, R. L. FERGUSON, Department of Pathology, University of South Dakota. (Introduced by Walter L. Hard)

Groups of weanling male rats were placed on synthetic diets lacking completely either phenylalanine, methionine or threonine.

The absence of any one of these essential amino acids resulted in atrophy of the testes, seminal vesicles and prostate, being most severe in the threonine deficient rats. Testicular and prostatic damage resulting from methionine deficiency, while severe, was not as extensive as in the other groups.

Alteration of the anterior pituitary cells occurred as a result of the three deficiencies studied. A lack of phenylalanine resulted in a decrease in acidophil size. The basophils, while not appearing reduced in number or size, were less intense in staining reaction.

Methionine deficiency also resulted in a decrease in acidophil size. The basophils of the pituitaries of these animals appeared enlarged and some vacuolated basophils were apparent. These cells resembled castration cells.

Absence of threonine from the diet resulted in a decrease in number of both types of chromophilic cells of the anterior pituitary and in extensive cellular damage to this gland. The degenerate cells were completely lacking in cytoplasm with only remnants of cellular and nuclear membranes remaining. Because of the extent of cellular damage, it was impossible to identify the cells involved in this degeneration.

¹ This work was supported by a grant from the United States Public Health Service.

123. *The adepidermal reticular network of the skin of the urodele, Triturus.*

Marcus SINGER and Jean S. ANDREWS, Department of Anatomy, Harvard Medical School.

Studies of the skin of the newt with Pap's ammoniacal silver method (as modified by Mitchell and Wislocki, '44) revealed a unique reticular structure of the "basement membrane." In cross-section the membrane appeared as a thin fiber containing frequent pronounced enlargements which gave it a beaded or nodal appearance. Both nodal and internodal regions were connected by occasional fibrils to the underlying dermal reticulum. The enlarged beaded region

was also quite apparent in tangential view, but the internodal zone was quite different and consisted of fibrils radiating from a node as the center. The nodal structure and the fibrils which appeared to emanate from it gave the appearance of spokes originating from the large central hub (the node) of a rimless and often eccentrically-shaped wheel. The fibrils diminished in diameter distally where they generally encountered and fused or interlaced with fibers radiating from similar and neighboring centers. These adepidermal reticular structures were seen in the skin of arm, hand, tail, snout, and belly of both adult and eft. Structures resembling closely those observed with the silver method were revealed by other fixing and staining techniques including acid dyes such as aniline blue, light green and eosin.

50. *Regeneration of sensory olfactory epithelium and nerves in adult frogs.* C. G. SMITH, Department of Anatomy, University of Toronto.

Regeneration of the olfactory epithelium of the frog was observed (a) after all the epithelium within one nasal cavity was destroyed by 1% zinc sulphate, and (b) after a small portion of the epithelium and its associated tunica propria were cut away. In (a) the new epithelium appeared to regenerate from surviving cells of Bowman's glands located in the tunica propria. In (b) all the epithelial cells bordering the wound appeared to share equally in the formation of the new epithelial cells. Regeneration is completed within 70 days.

The olfactory nerve fibers degenerate and disappear following the destruction of the olfactory sensory cells. During the third week new nerve fibers begin to reappear in the olfactory nerve and by the 70th day the section of the nerve, resembles the section of a normal nerve.

45. *On the source of birefringence in striated muscle.*¹ R. Dale SMITH, Department of Anatomy, Creighton University School of Medicine and William R. AMBERSON, Department of Physiology, University of Maryland School of Medicine.

Entire exsanguinated rabbit muscles were subjected to extraction with potassium pyrophosphate solutions at 0°C. for periods ranging from a few days to several weeks. This treatment resulted in the liberation of muscle proteins which, when examined electrophoretically revealed the presence of at least four distinct components. A prominent component (C) was identified as myosin while another component (B) of similar velocity remains unidentified. It was further observed that components B and C could and would combine to form a complex which reacted negatively to all tests for actomyosin yet showed definite flow birefringence. Such extracted muscles when examined histologically in ordinary light showed remarkably little change in structure. The fibers were contracted but the contraction bands were clear and distinct and appeared as the usual dense highly refractile areas. When the fibers were studied in polarized light they exhibited a marked reduction in birefringence. After a few days of extraction all of the fibers showed a general reduction in birefringence and at the end of a week only a faint trace of the original birefringence remained. The reduction in birefringence in the individual fibers was uniform and therefore entirely different from that seen

in rigor mortis. Muscles held at 0°C. in Ringer-Locke solution for similar periods of time showed no marked changes in birefringence. Since the extraction procedure removes proteins of sufficient dimensions to show flow birefringence and the extracted muscle shows considerable loss of its birefringence it might be presumed that these proteins are responsible for at least part of the birefringence of the intact muscle. It is also indicated that the striations of muscle as seen in ordinary light are not produced by the birefringent material.

¹ Aided by grants from the Bressler Alumni Research Fund and the National Institute of Health.

149. *Single unit activity in the spinocerebellar tract in cats.* James M. SPRAGUE, Department of Anatomy, School of Medicine, University of Pennsylvania.

Activity of single fibers in the lateral funiculus was studied, using steel micro-electrodes, electrolytically sharpened. Following movement of hairs, pressure on and stretching of muscles and tendons in hind leg and tail, and electrical stimulation of minute cutaneous and muscle nerve twigs in the hind foot, abundant activity was recorded from various electrode placements in the ipsilateral, lateral funiculus of segments T-8, 9 and 10. Responses to tactile and proprioceptive stimulation were present in deep and superficial parts of the lateral funiculus. Effective electrical stimulation was given by single, square-wave pulses.

The activity of a single central unit was frequently recorded from one electrode placement following both tactile and muscle-proprioceptive stimulation in the hind foot. It is not known whether this activity was from the same fiber in both types of stimulation. However, this type of activity in neighboring fibers was assumed to be relayed through a single nucleus, Clarke's nucleus, which projects ipsilaterally to the cerebellum. No contralateral activity was found at any depth in the lateral funiculus following stimulation of nerve terminals, twigs, large nerves, or dorsal roots. It is therefore concluded that the border cells of Cooper and Sherrington which have a contralateral projection to the brain stem and cerebellum via the lateral funiculus are not under the influence of activity coming from the periphery.

187. *Fixed tissue content of phosphorus compounds, and the radio-autographic localization of P^{32} .* C. E. STEVENS, Department of Anatomy, McGill University. (Introduced by J. Gross)

The liver, spleen and other tissues of rats given radio-phosphorus were fixed in Orth, 10% formalin and Carnoy fluids and prepared as radio-autographs. Chemical analyses and specific activity determinations were also performed on the fresh and fixed tissues. In this way the fixed tissue retention of acid-soluble and fat-soluble phosphorus fractions, phospho-protein, RNA and DNA were estimated in the hope of determining which phosphorus-containing compounds were responsible for the radio-autographic reactions.

The results of these analyses indicated a difference in the retention of phosphorus compounds in tissues after the three types of fixation. Orth fixation was found to retain most phosphorus containing substances and is the fixative of choice for the general demonstration of P^{32} in tissues. Formalin fixation retained somewhat

less, while Carnoy removed almost all of the acid- and fat-soluble phosphorus compounds.

In order to analyse further the radioactive material retained in the sections, they were treated with ribonuclease or desoxyribonuclease. It was possible, with Carnoy fixation and suitable enzyme treatment of sections, to demonstrate specifically the localization of RNA or DNA containing P^{32} .

153. *Further studies of the bulbar acoustic centers.* W. A. STOTLER, Department of Anatomy, University of Oregon Medical School.

By selectively destroying portions of the spiral ganglion in cats, degeneration has been produced in the ventro-lateral part of the ventral cochlear nucleus by apical lesions, and in dorso-medial areas of the nucleus by basal lesions. Lesions of the ganglion intermediate to these have produced corresponding degenerations in the nucleus. Total destruction of the cochlea and spiral ganglion has not produced discernible alteration of the neuropil of the tuberculum acousticum.

Lesions of the anterior segment of the ventral cochlear nucleus produced degeneration of the terminals on the proximal pole of the cells of the contralateral medial superior olivary nucleus, and complete degeneration of terminals on the homolateral lateral nucleus as well as disappearance of the calyces on cells of the contralateral trapezoid nucleus. Lesions of the posterior segment of the ventral cochlear nucleus produced degeneration of terminals to the contralateral nucleus of the lateral lemniscus.

Lesion of the lateral lemniscus at the base of the inferior colliculus produces chromatolysis of the cells of the medial superior olivary nucleus of the same side, and partial involvement of cells of the lateral nucleus of both sides. No alteration was produced in cells of the trapezoid nucleus.

Corresponding experiments with pigeons show that the nucleus laminaris of birds corresponds in morphology and connections to the medial accessory superior olive.

72. *The theca cone and its tropism toward the ovarian surface, a typical feature of growing human and mammalian follicles.* Erwin O. STRASSMANN, Department of Gynecology, Baylor University College of Medicine. (Introduced by G. Gordon Robertson)

The findings are based upon over 18,000 microscopic sections in four mammalian orders: Primates (man), Carnivora (dog, cat), Rodentia (rabbit), Ungulata (horse, cow, swine).

1. The diameter of human growing follicles and their distance from the ovarian surface were measured micrometrically. In the early stages up to a diameter of 0.25 mm, there is a descent of the follicles from the albuginea toward the hilus. There is an ascent of the larger follicles back to the surface. This ascension begins with the appearance of the theca layers.

2. Examination of the theca layers shows that their growth is an eccentric one. In serial sections cut perpendicular to the ovarian surface, there is a one-sided thickness of the theca interna, a wedge-like "theca interna cone" with a

triangular cut surface, which always points to the nearest part of the ovarian surface. The follicle proper follows the line provided by the theca cone. The granulosa frequently protrudes into the cone.

3. In all mammalian species with a free ovarian surface the theca cones grow divergently toward the next point of the ovarian surface. In horses where the ovaries are surrounded by connective tissue, the theca cones grow convergently toward the "ovulatory pit." This proves that the theca cone fulfills the purpose of bringing the follicle to that part of the ovarian surface where ovulation can take place.

97. *Early stages in the development of the mammalian choroid plexus of the lateral ventricles, as exemplified by the rabbit.* Leon H. STRONG and Richard A. COHEN, Department of Anatomy, The Chicago Medical School.

Embryos injected through the umbilical vein, while living, cleared in benzylbenzoate, and cut in 500 $m\mu$ sections, show the choroid plexus grossly.

The primitive internal carotid artery of the 11-day embryo forks rostrally and dorsally, at the junction of the tel- and diencephalon. From each fork a slender vessel passes to join a plexus concentrated in the choroid fissure and in continuity with the general plexus of the optic stalk. Rostrally this plexus feeds the concentration of capillaries in the midline, the future superior sagittal sinus. The lateral head vein also drains this plexus.

In the 12-day embryo the plexus of the fissure has reduced itself to one or two slender choroid arteries caudally. From the dorsal fork of the internal carotid at the level of the cephalic flexure, an artery joins the choroid artery at the caudal end of the plexus. The ependyma of the choroid fissure develops a free edge laterally.

In the 13th- and 14th- day embryos, the laterally grown ependyma develops two shelves, projecting laterally. Caudally they separate, rostrally they meet. The choroid artery vascularizes these shelves, by developing vessels around the periphery of the shelves, and a plexus between this vessel and the supplying arteries.

Three successive daily stages show, first, crenulated shelf borders, second shelf undulation, and third, villi.

113. *Effects of continuous irradiation on mitosis in tissue cultures.* Agnes N. STROUD, (by invitation) and Austin M. BRUES, Division of Biological and Medical Research, Argonne National Laboratory.

Chick embryo muscle cells have been cultivated *in vitro* and irradiated continuously by introduction of tritium oxide into the medium. Radiation dosage rates, calculated physically, were equivalent to 46 and 93 roentgens per hour. The duration and frequency of mitoses were estimated by photographing fields of approximately 100 cells at 10-minute intervals. The mean duration of mitosis was 33 minutes in control cultures, and in the first two hours of irradiation was 50 minutes at 46 roentgens per hour and 68 at 93 roentgens per hour. The degree of mitotic delay in irradiated cultures was highly variable, and the data suggest

different modes of delay. The rate at which irradiating resting cells entered mitosis was equivalent to that of controls in the first hour and declined thereafter; but the prolongation of the mitotic process did not seem to depend on the duration of prior irradiation.

177. *Connective tissue mast-cell responses to bacterial pyrogens, ovalbumin and cortisone.*¹ Edward G. STUART, Department of Anatomy, School of Medicine, University of Pennsylvania. (Introduced by Ruth Rhines)

A type response of the connective tissue mast cell of the mouse elicited after injection of certain bacterial pyrogens differs from that produced by chemically dissimilar substances. Pyrogens extracted from a *Pseudomonas* species, *Proteus vulgaris* and *B. subtilis* were injected intraperitoneally in single 10 gamma dosages into C-57 mice. Animals sacrificed in pairs on successive days thereafter showed the reaction varying in degree and time but not in basic pattern.

Since all these pyrogens are essentially polysaccharides, it was decided to test proteins and steroids. Accordingly, similar single injections of 10 mg of ACTH, DCA and cortisone were made. ACTH and DCA elicited only suggestions of a response. Cortisone produced a marked reaction which differed in its morphological characteristics from that of the pyrogens used.

Ovalbumin in single 1 mg injections gave a picture which differed histologically from the preceding ones. *E. typhosa* concentrate in a single 10 gamma dose produced a pattern somewhat like that of ovalbumin.

The most striking difference in pattern was in the contrasting sequence of change of the mast cells of two locations: those of the vascular adventitia and those of the intervascular areas. Alterations in granulation, pyknosis of some and expansion of other cells, varying distribution and arrangement of cells and cell groups, and cellular deterioration with concomitant release of granules furnished the basis for characterizing types of response elicited by these substances.

¹ Aided by a grant from Baxter Laboratories, Inc.

109. *Dextran in cultures of embryonic skeletal muscle.* Josel SZEPSENWOL, Department of Anatomy and Winship Clinic, Emory University School of Medicine.

It has been demonstrated that an excess of glycogen or glucose may completely inhibit spontaneous activity of embryonic skeletal muscle in tissue culture. When glycogen in a relatively high concentration is added to the culture medium it is in part hydrolyzed to glucose (probably by plasma amylase) and in part broken down to lactic acid. The glucose increases considerably in the coagula, even in the absence of explants. The increase in lactic acid, which may reach twice the control level, occurs only in the presence of explants. Of the glycogen present in the culture only a small fraction is extractable with trichloroacetic acid, the total polysaccharide can be extracted only with KOH.

In an attempt to find out whether the above action of glycogen is specific, the effect of other carbohydrates was studied. It was found that Dextran (also a

polysaccharide but with a different orientation of the glucose molecules, kindly supplied by Commercial Solvents Corp.) in concentrations of 1-1.5% has no effect upon the spontaneous activity of embryonic skeletal muscle, and no toxic effect upon growth. Chemical analysis shows that Dextran is not used by the explant; its concentration remains unchanged throughout the six days of culture. Glucose and lactic acid concentrations in these cultures are the same as in the controls. Moreover, the Dextran, unlike the glycogen, is all extractable with trichloroacetic acid, suggesting that it remains free.

75. *An investigation of the role of estrogen and luteotrophin in the development of large corpora lutea in the hypophysectomized rat.* George B. TALBERT, Department of Zoology, University of Wisconsin and Department of Anatomy, State University Medical Center at New York City. (Introduced by Roland K. Meyer)

This study was carried out on the corpora lutea of lactation in young adult rats. The animals were divided into 5 groups. The rats in 4 of these groups were hypophysectomized on the 2nd day and autopsied on the 17th day following parturition. The fifth group consisted of normal controls. One of the groups of hypophysectomized rats was injected daily with 34 gamma of diethylstilbestrol; the second group received 10 I.U. of luteotrophin daily; the third group received both diethylstilbestrol and luteotrophin; and the fourth group was untreated. All injections were given from the second through the 16th day of lactation.

At autopsy the corpora lutea were dissected and weighed with the exception of one ovary from each group, which was prepared for histological examination.

The results of this study showed that the corpora lutea from rats which were hypophysectomized and not injected and those which received diethylstilbestrol only were comparable in weight and showed little or no growth from the time of hypophysectomy.

The corpora lutea from the animals injected with luteotrophin were approximately the size of those found in the normal control animals but the rats receiving estrogen and luteotrophin were considerably larger.

These results indicate that diethylstilbestrol, although ineffective alone in inducing growth of corpora lutea, may augment the growth producing action of luteotrophin.

14. *Arteriovenous shunts and blood supply of the bronchi in isolated, injected human lungs.*² Charles E. TOBIN and Manuel O. ZARIQUIEY, Departments of Anatomy and Radiology, The University of Rochester School of Medicine and Dentistry, and the Medical Section, Eastman Kodak Company.

Studies were made of the blood vessels in 25 normal lungs, obtained from autopsy, and in 10 pathologic lobes, from surgery, autopsy or cadavers; with age ranges from newborn to 90 years. After perfusing the specimens with saline solution via the pulmonary artery, the following vessels were injected with the media specified for the number of cases: pulmonary artery—glass spheres, 10-750 micra in diameter (27), radiopaque media (6), followed by vinyl ace-

tate (25), or liquid latex (10); pulmonary veins—vinyl acetate (27), or liquid latex (8); and bronchial arteries—vinyl acetate (8), or liquid latex (4). The specimens were radiographed, macerated, dissected or sectioned.

These studies demonstrated that: 1—shunts between the pulmonary artery and veins were in the lobules (20–500 micra) and in the visceral pleura (20–200 micra), as shown by the passage of the spheres from the pulmonary artery to and through the pulmonary veins. The locations of the shunts were determined from the roentgenograms and from the latex or vinyl acetate injected specimens. 2—The bronchial and pulmonary arteries, with anastomoses between these arteries, were observed to supply the intrapulmonary course of the bronchi and the mediastinal visceral pleura. As reported by others, in certain diseased conditions or in the newborn these anastomoses may be large; anastomoses 400–600 micra in diameter were observed in such cases.

¹ Aided by a grant from the Fluid Research Fund, The University of Rochester School of Medicine and Dentistry.

16. *Some interesting veins of the heart.*¹ R. C. TRUEX and M. J. SCHWARTZ,
Division of Anatomy, Hahnemann Medical College.

Clinical investigators have observed a temporary common bundle block in hearts of children with diphtheria. Similar findings have been reported in serum sickness. While studying the atrial and ventricular portions of the conduction tissue of newborn and adult hearts (man, dog) we have consistently observed large venous channels in the atrio-ventricular area which may be related to the common bundle block seen in the above toxic diseases.

The veins arise in the posterior portion of the interventricular septum and adjacent myocardium of the left ventricle. These large sinusoidal channels ascend in the subendocardial region and cross the fibrous ring either within, or intimately related to, the common bundle of conduction tissue. Such dilated vessels anastomose freely as they follow longer or shorter courses in the common bundle and to a lesser extent in the atrio-ventricular node itself. Superiorly these sinusoids terminate in the coronary sinus, right atrium, or both. Reconstructions and serial sections suggest that the thin-walled sinusoids may play an important role as avenues for the dissemination of toxins into the common bundle in man, and thus explain the unusual electrocardiographic changes observed.

¹ This investigation supported by Research Grant H-321, from the National Heart Institute, United States Public Health Service.

194. *The distribution and excretion of some soluble salts of carrier-free radioberyllium.*¹ C. D. VAN CLEAVE, Department of Anatomy, University of North Carolina.

This work is part of a radioautographic investigation of the distribution of carrier-free Be7 in the rat. This primary purpose necessitated the injection of Be7 at high levels of activity. The distribution and excretion of 3 soluble salts of radioberyllium following intravenous, intramuscular and intraperitoneal injections were determined by a wet-ashing assay.

Intravenous Be^*Cl_2 and Be^*SO_4 localized chiefly in liver, spleen and skeleton: e.g., 6 millicuries of Be^*SO_4 gave maximum spleen activity (11.9%/gm) in 24 hours, maximum liver activity (7.9%/gm) in 72 hours. Skeletal activity increased later at expense of liver and spleen. Of the total dose 13% or more was excreted in 24 hours; excretion dropped sharply thereafter.

Radioberyllium as the citrate injected by any route concentrated chiefly in skeleton, although about 70% of the dose was excreted in the first 24 hours.

Only Be^x citrate was completely mobilized from the site of injection following intramuscular injections. When Be^*Cl_2 (pH 2.0) was similarly injected 98% or more of the dose was recovered by assay from the muscular site; with Be^*SO_4 (pH 4.5 and 1.0), 80–90% was similarly recovered.

The results seem consistent with known solubility and colloidal properties of Be and confirm grossly the radioautographic findings.

¹ This work done under contract No. AT-(40-1)-266 for the U. S. Atomic Energy Commission.

48. *The influence of certain compounds on sound induced seizures in mice.* E. M. VICARI, A. TRACY and A. JONGBLOED, R. B. Jackson M. Laboratory.

Eight different compounds were tested in an effort to protect against the onset of convulsions in an inbred strain of mice at the age normally susceptible to sound induced convulsions.

The relationship of age to susceptibility was previously established by Vicari with 5012 mice as controls. The normal age susceptibility of the dilute brown strain is in the range of 20 to 80 days. The curve is bimodal with the highest frequency of 95% susceptibility at 25 to 30 days. The second peak is at 60 to 69 days with 20% susceptibility.

The present experiment involves 352 mice of known ages in the 28 to 39 days range which is normally about 95% susceptible. Compounds were administered subcutaneously, except thiouracil which was given in the food. Almost complete protection was found with epinephrine (one dose of 100 gamma). Epinephrine mixed with glucose gave 90 to 95% protection, and in order of less protection, thiouracil, glucose alone, testosterone, pregnenolone, cortin with 34% protection and estradiol with 32% protection.

96. *Observations on anencephalic human monsters.* Herbert S. WARREN, Division of Anatomy, Hahnemann Medical College. (Introduced by A. W. Angulo)

In a series of 9 acranic, anencephalic human monsters, two presented total rachischisis with complete amelia; and 7 specimens had spinal cords which were smaller in the transverse diameter than control cords. Two specimens had rudimentary medullary plates in the posterior cranial fossae. A meningocele of varying size was noted in each specimen. Completely developed eyes with attached optic nerves were present in all specimens, yet histologic sections of the mass of connective tissue and blood vessels beneath each meningocele failed to reveal any evidence of a cerebral hemisphere or brain stem. It is

assumed that a forebrain vesicle once existed, suffered developmental arrest, and failed to complete neural differentiation. The medullary plates observed in two specimens appear to represent undifferentiated cervical or upper thoracic segments of the spinal cord. Cortico-spinal tracts were absent in each of the 7 spinal cords, although spinal nerves and spinal ganglia were present. A consistent correlation was noted between spinal cord development, dorsal arches of the vertebrae, and dorsal axial musculature. Additional anomalies in 4 of the 9 specimens included: dorsal lumbar and diaphragmatic herniae, imperforate anus, hypertrophy of the thyroid gland, and total absence of external genitalia.

163. *On the interpretation of differences in the form of the skull.* S. L. WASHBURN, Anthropology Department, University of Chicago.

Differences between mammalian skulls associated with age, sex, or species have usually been described in terms of bone alone. This is reflected in gross anatomy text books in which much of the section on osteology has meaning only if the form of bone is independent and if individual bones are the most useful units of description. Experiments on rats have indicated that many features of the adult rat's skull are determined by the muscles and the interrelation of one bone to another. To relate these findings more closely to the situation in man, the temporal and masseter muscles of one hundred monkeys were removed and weighed. The weights were correlated with age, sex, and specific differences. The results show that changes in many parts of the skull (nose, orbit, palate, zygomatic arch, parts of the mandible, basal length and width, temporal and nuchal crests) are completely determined by, or highly correlated with, the size and proportions of the muscles. It is concluded that any description of the skull which is intended for analytical use must include a statement of muscle size, pattern, and bone-muscle relationships.

129. *Thyroid changes in guinea pigs which were goitrous at birth.* Richard C. WEBSTER, Department of Anatomy, Indiana University School of Medicine. (Introduced by Richard L. Webb)

In a previous abstract (American Society of Zoologists, 1948), it was reported that female guinea pigs fed thiouracil and mated with normal males gave birth to a sizable proportion of living offspring. The thyroids of these animals were greatly enlarged. Glands removed from 36 newborn individuals averaging 3491 mg, compared to 35 mg in normal newborn.

The follicles of these thyroid glands were extremely disorganized and colloid was absent. There is a gradual regression in size of the thyroid glands of animals allowed to mature. By the fourth day following birth, the first trace of colloid appears and the follicles have assumed a more characteristic appearance. At 14 days, colloid is abundant and the follicles are lined by cuboidal cells. In animals sacrificed 8½ months after birth the thyroids averaged 708 mg as compared to 149 mg in normal animals. The follicles were larger than normal and were lined by squamous epithelial cells.

The results obtained would seem to indicate that no permanent damage to the thyroid results from the hyperplasia induced during the fetal period. In animals allowed to mature, growth curves were similar to those of normal animals, and there was no delay in sexual maturation in either males or females.

23. *Extension of observations on the production of androgen by the testes of fetal rats.* L. J. WELLS and Rachel L. FRALICK, Department of Anatomy, University of Minnesota.

We had found that castration retards the growth of the seminal vesicle and bulbourethral gland in all cases and causes atrophic changes in the ductus deferens in most cases, and that all these effects may be prevented by implanting crystalline androgen.

In extending the work, unilateral castration and "sham" castration failed to retard the growth of the bulbourethral glands, organs which were not near the field of surgery. On the operated side of the body of 9 unilaterally castrated fetuses the seminal vesicle was sometimes defective in lacking a flexed portion (3 cases), and the ductus deferens was sometimes atrophic (4 cases). It seems likely that these changes were caused by an impairment of the deferential circulation; the deferential artery contributes blood to the seminal vesicle, and might have been surgically injured, or ruptured, in a number of cases. This view is supported by the absence of such changes in "sham" castrated fetuses and by the lack of retarded growth of control organs in bilaterally castrated fetuses (kidneys and brain).

Accordingly, we do not join certain workers in postulating that the testicular secretion mostly acts locally or unilaterally. Although the evidence that the fetal testis produces a hormone (androgen) is virtually conclusive, an attempt to prevent the effects of castration by giving castrated fetuses testicular grafts has not been successful (abstract 238).

205. *Changes accompanying aging in the brains of guinea pigs.* Harry H. WILCOX, Department of Anatomy, School of Medicine, University of Pennsylvania. (Introduced by C. N. Liu)

The brains of guinea pigs ranging from 139 to 2765 days (7.6 years) old were provided by Dr. J. B. Rogers and Dr. C. A. Woerner. Perfusion-fixation with isotonic gum acacia solutions of formalin was followed by serial sectioning and staining with buffered thionin and other techniques. The most striking age change was accumulation of lipoid pigment in cells of various brain stem nuclei. The earliest deposits of pigment occurred in the mesencephalic nu. V and motor nu. XII. The red nuclei, motor nu. III, IV, VI, VII, and vestibular nuclei gained pigment slightly later. The cochlear nuclei and the dorsal motor nu. X seemed to be the last to undergo pigmentation. Changes in amount and distribution of Nissl substance were not observed at any age, except in cytoplasmic areas occupied by the lipoid pigment. No appreciable loss of neurons could be detected qualitatively. The chorioidal epithelium in the oldest animals

contained much melanin-like pigment, and fat accumulated within the connective tissue of the plexus. Changes in aged guinea pig brains prepared by the perfusion-fixation technique were less pronounced than those described in other species and in guinea pig brains fixed post-mortem by immersion in formalin.

93. *Vital and in vitro staining of dense connective tissue of the mouse by trypan blue.* W. Lane WILLIAMS and Richard H. SWIGART, Department of Anatomy, University of Minnesota.

Mice received subcutaneous or intraperitoneal injections of 0.5 to 3 mg of dye daily for one to 7 days, and were killed 30 minutes to 48 hours after one or more injections. Tendon, ligament, sclera, dermis, dura, periosteum and perichondrium were rapidly and intensely stained. Synovial linings, epiphysal cartilages, and articular surfaces of articular cartilages were less heavily stained. Other cartilage, bone and muscle were not stained. Trypan blue (0.25% solution in saline) in vitro stained tendon but not adjacent muscle. Similar reactions occurred when routine histological sections were stained in 0.1% trypan blue at pH 7. At pH 1-2 muscle was stained but not tendon. Fibrous tissue staining vitally with trypan blue also showed intense acidophilia (stained in ponceau 2R at pH 1-2) and a strong reaction to periodic acid-Schiff's reagent. These reactions were reduced by prior enzymatic (pepsin and trypsin) hydrolysis of sections. In vitro incubation of vitally stained tendon in a solution of trypsin released a large amount of the vital stain. Hyaluronidase removed a very small amount of dye. The cytochemical staining reactions plus the effects of enzymatic hydrolysis suggest that the vital staining resulted from binding of trypan blue by a protein-carbohydrate constituent of dense fibrous tissue.

4. *The necessity of normal architecture of the tissues for the functioning of the macrophages in the liver.*¹ J. Walter WILSON, Department of Biology, Brown University.

Colloidal thorium dioxide (Thorotrast), injected intravenously, is taken up by the macrophages of the liver and spleen. Injected into mice with hepatomas produced by repeated doses of CCl_4 , or prolonged feeding of o-aminoazotoluene, or occurring spontaneously in the C_3H strain, it is not taken up in the hepatoma. Histologically, the hepatoma resembles the liver parenchyma, with similar cells arranged in cords with sinusoids between them. The lobular architecture is, however, absent. There are no bile ducts, and bile capillaries cannot be demonstrated between the cells. Hepatomas have a predominantly arterial rather than portal blood supply (Breedis and Young, '49). If there are macrophages in the walls of the sinusoids they are inactive, since they fail to take up Thorotrast. We have previously reported evidence suggesting that the macrophages of the liver are not necessarily permanently fixed in the walls of the sinusoids. It is, therefore, suggested that there is a necessary relationship between the structural elements of the liver, involving the portal venous blood supply, and the connection

of the liver cells with the bile drainage system, in the absence of which the macrophages either fail to enter the walls of the sinusoids, or at least fail to function as such there.

¹ This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

5. *Neoplastic growths induced by x-irradiation in embryonic rats.*¹ James G. WILSON, Department of Anatomy, University of Cincinnati.

Rat embryos in the 9th day of gestation were irradiated directly through an abdominal incision in the mother. Lead plates were so arranged that only selected implantation sites were exposed while the mother and remaining sites were shielded. After post-irradiation intervals of one to 6 days the embryos were removed and prepared for histologic study.

Abnormal growths consisting of compact masses of epithelioid cells appeared in the mesenchyme surrounding the developing brain within two days after exposure to x-rays. Following an initial spurt of growth, some of these masses dispersed and were no longer recognizable, others remained compact but showed little further growth, and still others continued to grow rapidly, resulting usually in prenatal death of the embryo. Growth of the tumors beyond a minimal size appeared to be dependent upon the abundance of blood supply in the vicinity.

These embryonic neoplasms are believed to have arisen from certain cells within the neural primordium that were altered by the irradiation. This is supported by the following observations: (1) the occasional retention of a connection with the neural tube, (2) their histologic resemblance to contemporary tissues within the neural tube, and (3) a tendency to differentiate toward definitive neural elements.

The incidence of tumors, both number of animals affected and number of growths per animal, increased directly as the dosage increased from 25r to 200r.

¹ Based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

185. *Studies of the effects of roentgen radiation on mouse liver.* Margaret E. WILSON, K. K. TSUBOI and R. E. STOWELL, Department of Oncology, University of Kansas Medical Center.

Strain A mice have been irradiated with roentgen radiation delivered to the liver region, the rest of the body being shielded. Doses up to 12,000 r were used (dose calculated for 1 cm depth within the animal). Irradiated animals lost weight at a rate nearly comparable to unfed animals. Therefore, to distinguish between the effects of fasting and irradiation, both experimental and control mice were fasted throughout the experiments. Livers were studied one, two, and three days after irradiation.

Irradiated livers weigh less, in relation to body weight, than control livers. Chalkley ratio measurements show a decrease in cell size, due principally to a decrease in lipid. Usually both nuclei and nucleoli increase in size, and the nucleo-cytoplasmic ratio is also increased. The fat in the fasted irradiated mouse liver is found in cells at the center of each lobule, while there is little or no fat

in the cells near the portal areas. This is in sharp contrast to the general distribution of fat in livers of fasted controls. Isolated necrotic cells and more extensive areas of necrosis and hemorrhage are found most often in the portal areas.

44. *The histology and cytochemistry of the basal plate and septa placentae of the normal human placenta delivered at full term.*¹ George B. WISLOCKI, Department of Anatomy, Harvard Medical School.

The basal plate and septa placentae of the human placenta at full term comprise sheets of fibrin in which numerous cells are embedded. Contrary to current opinion, these masses of cells are mainly fetal and not maternal ("decidual") in origin, and thus constitute a persistent trophoblastic shell. By ordinary histological methods trophoblastic and decidual cells are difficult to distinguish, but by histochemical means they can be readily told apart. On the one hand, the cytoplasm of the persistent, healthy trophoblasts contains much basophilic substance, periodic acid-Schiff positive (saliva-insoluble) stippling (possibly chorionic gonadotropin), many mitochondria (phospholipids), no neutral fat, variable glycogen, and practically no acid or alkaline phosphatase. The decidual cells, on the contrary, undergo considerable degeneration and reveal mild basophilia, a faint diffuse periodic acid-Schiff reaction (saliva-insoluble), much glycogen, neutral fat and acid phosphatase, and traces of alkaline phosphatase. The ground substance of the basal plate gives an intense periodic acid-Schiff reaction (fibrin), but is otherwise negative; contrariwise, the ground substance of the decidua basalis is metachromatic (acid mucopolysaccharide) and periodic acid-Schiff positive, in association with a collagenous reticulum.

The viable fetal cells persisting in the basal plate could account for the continuing production of gonadotropin. The trophoblastic Langhans cells of the chorionic villi, which disappear by mid-pregnancy, are characteristically chromophobic and thus quite unlike the related chromophilic cells of the trophoblastic shell and plate.

¹ Supported in part by an institutional grant from the American Cancer Society.

65. *The development of the bronchial columella in the aquatic ear of Ranidae.* Emil WITSCHI, Department of Zoology, The State University of Iowa.

Within the Amphibian class the ear acquires two structures of importance for the evolution of the terrestrial organ. (1) The basilar papilla, present in all but the most primitive urodeles, is the rudimentary form of the cochlea. (2) The tympanic organ, present only in anurans, reaches the highest development in frogs.

In the past, the underwater-hearing of amphibian larvae received but little attention. Our recent observations show that in some tadpoles the airfilled lung sacs are made use of as resonators. In the primitive Alytes the perilymphatic duct forms a prolapse through the round window of the otic capsule, approaching the bronchus of the same body side. In *Xenopus*, each bronchus develops a diverticle that ascends behind the chondrocranium and applies itself to the round window. The highest perfection is reached in the frogs. Immediately after

closure of the gill sacs, the suspensory ligament of the seventh pharyngeal pouch (lung bud) becomes fibrous and its attachment moves into the center of the round window. The dorsal aorta, shifting medially, soon engulfs the ligament, which thus becomes free of tissue attachments. At the surface of the bronchus this columella divides into three branches, two of which encircle a dome-shaped bronchial membrane. During metamorphosis the bronchial columella becomes completely reduced. These developmental processes will be illustrated by color slides.

145. *Endothelial reaction to intravenous lipids.*¹ Charles A. WOERNER, Department of Anatomy, University of Louisville.

Lipids which may be deposited in the intima of arteries were injected intravenously into guinea pigs. For this study triglycerides as cotton seed oil, mixtures containing lipoproteins as egg yolk and other lipids as cod liver oil, linseed oil, and lanolin were used. The emulsions were prepared in a high pressure homogenizer and stabilized with suitable substances, so that the droplet size would be less than 1 micron. As much as 10 grams of cottonseed oil per kilogram of animal, 1.5 grams of cholesterol, in colloidal solution, and from 5 to 10 grams of the other lipids per kilogram of animal were injected in 24 hours.

When cottonseed oil emulsions were injected no lipids were found in the endothelium or intima of the arteries studied. After the injection of colloidal solutions of cholesterol only occasionally were small droplets of lipid found in the endothelium. When emulsions of complex lipids as egg yolk were injected the lipid droplets in the endothelium were larger, more numerous, and more widely distributed.

¹ Aided by a grant from the National Heart Institute (H-180).

19. *The arteries of the pancreas.* Russell T. WOODBURN and (by invitation) Lloyd L. OLSEN, Department of Anatomy, University of Michigan Medical School.

Two arterial arcades characterize the anterior and posterior surfaces of the head of the pancreas. The gastroduodenal artery divides into the right gastroepiploic and anterior superior pancreaticoduodenal arteries, the latter contributing to the anterior pancreatic arcade. A posterior superior pancreaticoduodenal artery arises from the retroduodenal portion of the gastroduodenal artery and contributes to the posterior arcade. The arcades are completed by posterior inferior, and anterior inferior pancreaticoduodenal arteries which arise from the upper end of the superior mesenteric system, most commonly (in about 50% of over 100 dissections) by a common stem from that artery. A dorsal pancreatic artery occurs in almost three-quarters of the specimens. It arises from the celiac axis (50% of its total occurrence) or from the first 1.5 cm of the splenic or hepatic artery, and descends dorsal to the pancreas and splenic vein. An inferior pancreatic artery courses along the dorsal inferior aspect of the body of the pancreas as a virtually constant vessel but is formed from various sources, notably the left (or transverse pancreatic) branch of the dorsal pancreatic artery and left

branch of the anterior superior pancreaticoduodenal artery. The "arteria pancreatica magna" occurs in approximately one-half of the dissections and caudal pancreatic branches of the splenic artery to the tail of the pancreas in three-fourths of the specimens.

182. *Selection of sucrose solution by Yale and Wistar Rats.*¹ E. H. YEAKEL, Division of Anatomy, Hahnemann Medical College.

The Yale strain of rat is known to exhibit inherited diabetic tendencies. Since the choice of certain dietary components may be affected in the rat by various metabolic defects, it was decided to investigate and compare the appetites for sucrose of the Yale and Wistar strains. Both tap water and 5% sucrose solution were offered to the experimental animals. After 3 months on this regime, the following was observed: (1) In the control group with only tap water available, Wistar rats consumed 27 cc per day, and the Yale rats almost twice as much (50 cc). (2) When sucrose solution also was available, no tap water was taken by rats of either strain. (3) The sugar solution intake per day of the Wistar rat was 139 cc, a 500% increase over the fluid consumption of the controls. (4) The intake in the Yale rats was 123 cc per day, a 250% increase over the control value. Although both Yale and Wistar rats showed a preference for sucrose solution to the complete exclusion of water, the appetite of the Yale rats for sucrose was less than that of the Wistar rats. This self-restriction on the part of the Yale rats may be interpreted as an expression of their diabetic tendencies.

¹ This investigation was supported by a research grant from the National Heart Institute of the Public Health Service.

147. *Topographic localization within the dorsal spino-cerebellar tract.* Robert E. YOSS, Department of Anatomy, University of Michigan. (Introduced by Elizabeth C. Crosby)

In a series of 11 macaques, lesions of the spinal cord were produced at lumbar, thoracic, and cervical levels. After staining by the Marchi technique, the degenerated fibers in the dorsal spino-cerebellar tract were then studied with especial regard to any pattern of localization within this tract.

Sherrington and Laslett ('03) first demonstrated in the dog a pattern of localization within the dorsal spino-cerebellar system. This pattern was primarily one of simple stratification, with the more rostrally arising fibers of the cord joining the tract on its medial surface. Other investigators have confirmed this pattern in various animals.

Indications are, however, that there is a different pattern of localization within the macaque dorsal spino-cerebellar tract. The fibers from the upper lumbar region first shift laterally to the periphery of the lateral column and form a layer of fibers on the surface of the cord in the lower thoracic region, with some fibers extending dorso-medially between the posterior horn and the pyramidal tract. As these fibers ascend in the thoracic cord, there is a gradual shift dorsally.

These fibers of lumbar origin are all located in a compact bundle dorsal to the pyramidal tract in the cervical region. Fibers from successively higher levels first shift laterally and then dorsally to positions ventral to the more caudally arising dorsal spino-cerebellar fibers.

162. *Anatomical and functional relationships between the jaw and the ear.* M. Wharton YOUNG, Department of Anatomy, College of Medicine, Howard University.

There is much clinical evidence that indicates a functional relationship between the dental apparatus and the ear (Costen's syndrome). The anatomical basis for this interrelationship is still obscure. In evolution, the ear develops progressively closer to the jaw and in mammals the ancestral jaw elements are utilized as ossicles. In the embryo the otic vesicle develops dorsal to the mandibular arch but the mesenchyme of these two areas is continuous. Within this primordial tissue the first otic lymph channels are developed. The continuity and the extent of this mesenchyme is emphasized by the chondrification of Meckel's cartilage, extending from the periotic to the mandibular regions via the embryonic petrotympanic fissure. The lymph channels follow the same route, and those in the tegment tympani traverse the adult Glaserian fissure then continue their course in the soft tissues of the mandibular fossa where they are subject to compression by a displaced condyle. Such obstruction to the outflow of lymph with resulting labyrinthine dysfunction could explain the otomandibular complex.

The presence of these adult lymph channels has been established by intralabyrinthine dye and roentgenographic studies, by casts of the interior of the temporal bone and serial sections. Experimental cauterization of the mandibular fossa, in macaques, resulted in circumscribed areas of new bone formation accompanied by a marked increase in the alkaline blood phosphatase.

40. *A correlated histochemical and biochemical study of the influence of age on mouse liver alkaline and acid phosphatases.* Anita ZORZOLI, Department of Biochemistry, Washington University Dental School.

The alkaline and acid phosphatase activities of the liver of C 57 Black (subline 6) mice are being studied as a function of the growth and aging of the animals. With the exception of the very early ages where several livers are pooled, two samples are taken from each liver. One is homogenized and used for a quantitative estimation of the enzyme activities and the total protein content; the other is fixed in acetone and treated by the methods of Gomori for the histochemical localization of the alkaline and acid enzymes. Sodium glycerophosphate is used as the substrate in both methods.

Data obtained at monthly intervals from the 27th postnatal day to one year of age indicate that there is a remarkable constancy in both the localization and degree of alkaline phosphatase activity. The acid enzyme exhibits more variability both within each age group and between these groups. However, none of the differences approach statistical significance. From the 2nd to the 20th post-natal days there is a significantly higher activity of both the alkaline and acid phosphatases than in all the older age groups.

PAPERS READ BY TITLE

- 206a. *Influence of hypertonic injections on the number of white blood cells.*
Rudolf ALTSCHUL, Department of Anatomy, University of Saskatchewan.

Intravenous injections of hypertonic solutions (10 cm³ of 50% glucose; or of 50% sucrose; or of 20% calcium gluconate) in normal rabbits are followed by a relative decrease of lymphocytes and a relatively increase of neutrophils, but the total WBC and RBC counts show little if any variations. Approximately 12 hours after the injection the blood cell proportions have again returned to the pre-injection pattern. These variations in the cell proportions might be due (a) to the so-called "distribution leucocytosis," (b) to the "stress" phenomenon or (c) to changes in the WBC formation, owing to the influence of hypertonic solutions on mitosis. Control examinations show that a similar but less marked alteration in WBC follows the simple takings of blood.

There are some indications that significant changes in the relative numbers of neutrophils and lymphocytes can be induced in disease by intravenous injections of hypertonic solutions, particularly in various forms of leukaemia. These possibilities are being examined.

Supported by a grant from the National Research Council of Canada, Division of Medical Research.

207. *Cell changes in the islets of Langerhans in rats treated with adrenocorticotropin.* Burton L. BAKER, Marjorie A. SCHAIRER, Dwight J. INGLE and Choh Hao LI, University of Michigan, The Upjohn Company, and University of California.

Since administration of adrenocorticotropin to rats at sufficiently high doses will cause hyperglycemia and glycosuria, it may be expected that the function and structure of the islets of Langerhans would be modified. Differential cell counts were made on the islets of rats so treated, the sections being fixed in Bouin's fluid and stained by Gomori's phosphotungstic acid-phloxine procedure. Many alpha cells were degranulated. High doses were required to produce changes in the beta cells. When 6 mg was given daily by continuous injection for 21 days the islets were enlarged and new ones formed. In exceptional cases the majority of the beta cells were degranulated. Hydropic degeneration has not been observed. It is believed that these evidences of hyperactivity are secondary to the changes in carbohydrate metabolism induced by adrenocorticotropin.

208. *The distribution of metachromasia in the heart of the embryonic chick.*
Alexander BARRY, Department of Anatomy, University of Michigan Medical School.

The work here presented is part of an investigation of the chemical composition of the wall of the embryonic heart, with particular reference to the endocardium. Chick embryos of from two to three days of incubation were fixed in 4% aqueous hydrated lead acetate at 3°C. for 36 hours after which they were embedded in paraffin and serially sectioned. The sections were stained with 1% aqueous toluidine

blue and mounted from water in 20% levulose. This technique stains polysaccharides and mucoproteins metachromatically. The cardiac jelly in these preparations was in the form of a reticulum of coarse and fine strands. In some regions the strands were so delicate as to make the individual fibrilli indistinguishable. These strands were confined to the endocardium, and were the only structures in the heart that stained metachromatically, making it probable that they were precipitated polysaccharides or mucoproteins. The metachromatic material was found consistently in the cardiac jelly of the truncus, conus, and ventricle. In addition a thin, intensely staining layer extended from the cardiac jelly of the atrio-ventricular canal between the endothelium and the myocardium of the caudal part of the atrium. No metachromasia was found in the sinus venosus or that part of the atrium adjacent to it. Work is in progress to determine the identity of the metachromatic staining material.

209. *Histological studies on the effects of intrathecal procaine in the monkey.* Kenneth R. BRIZZEE, Dana-Lee HARNAGEL and Scott M. SMITH, Departments of Anatomy and Anesthesiology, College of Medicine, University of Utah.

Preliminary to an investigation of the potentiating and detoxifying effects of certain drugs administered intrathecally with spinal anesthetics the minimum lethal dosage of intrathecal procaine and the histological changes in the spinal cord following various dosages of the drug have been investigated.

Doses greater than 7.5 mg/lb. in 3% solution proved lethal within 30 minutes in 6 animals. From clinical signs and symptoms the cause of death appeared to be systemic rather than histotoxic.

One animal receiving 10 mg/lb., in extremis 30 minutes after injection was sacrificed and perfused with 20% formalin solution and cord tissues were stained with buffered thionin and galloxyanin. Histologically these tissues did not differ from those of a normal intact animal or a saline control animal perfused 48 hours after injection.

One animal of three which received 7.5 mg/lb. survived. Tissues from this animal were fixed by perfusion 48 hours after the injection. These tissues showed widespread and severe chromatolytic changes in the lumbosacral region of the spinal cord but these alterations were considerably less marked in the cervical region.

Myelin degeneration was demonstrated in the posterior funiculus of the cord in Swank-Davenport preparations of lumbar, thoracic, and cervical regions, decreasing in extent at the higher levels.

In two animals receiving 6 mg/lb. the tissue alterations were much less severe, and in two receiving 5 mg/lb. were relatively slight. One animal which was given 1 mg/lb. in 5% solution showed no greater alterations than the latter two.

210. *Growth response of the chick nervous system after the transplantation of mouse rhabdomyosarcoma in the hind limb field.*¹ Elmer D. BUEKER, Department of Anatomy, University of Missouri.

Mice (C3H strain) with rhabdomyosarcoma were obtained from the Lankenau Hospital in Philadelphia through the courtesy of Elizabeth Ufford Green. Tumor

fragments were transferred to 48-60 hour chick embryos and wedged in longitudinal incisions in the somatopleure lateral to somites 20-30. Of 25 embryos, 20 survived which were sacrificed at 5-10 days incubation and sectioned.

At sacrifice transplants were located near the base of lower extremities and were accessible to invasion of outgrowing nerves. Nevertheless, peripheral nerves of 5-9 day cases usually extended around tumors or penetrated short distances and ended abruptly. Eighty to 90% of the anterior horn cells were missing; sympathetic ganglia were smaller and total volume reductions of spinal ganglia of different cases varied from 18-32%.

In one 10-day case total volume reduction of 8 ganglia adjacent to the tumor was 3% less than controls; four of these ganglia were larger than controls. Nerve fibers from the enlarged ganglia were traced to the sciatic nerve which penetrated deeply into the tumor. In the four 9-day animals the occurrence of enlarged ganglia was not unusual. Previous observations suggest, (Bueker, '48, Levi-Montalcini and Hamburger, '50) that the enlarged spinal ganglia may have been caused by the sarcoma component of this tumor.

¹ Aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health.

211. *The ineffectiveness of the administration of hyaluronidase in altering hair growth in the rat.* Earl O. BUTCHER,¹ Departments of Anatomy, College of Dentistry and The Graduate School of Arts and Science, New York University.

Since bilateral adrenalectomy (Butcher, '37) induces an earlier growth of hair in the rat, and there is increased dermal permeability following adrenalectomy (Opsahl, '49), it seemed interesting to learn if local treatment of the skin with hyaluronidase would alter growth.

Accordingly, hyaluronidase was administered daily to rats from the 22nd to 29th days of age in the following ways: (1) seventy to 280 turbidity reducing units in normal saline were injected either intradermally or subcutaneously into a dorsal area; (2) 7000 T.R.U. were incorporated in 1 gm of carbowax (1500) and some of this combination was applied to a surface area on the back; (3) 700 to 1400 T.R.U. in peanut oil were injected intradermally or subcutaneously into a dorsal area.

There was no alteration of the time of growth in any instance. The accelerated hair growth following adrenalectomy in all probability is not entirely a matter of dermal permeability.

¹ The hyaluronidase was kindly supplied by Dr. Seifter of the Wyeth Institute.

212. *Decapitation of chick embryos during the latter half of the incubation period.* J. F. CASE, Department of Biology, The Johns Hopkins University. (Introduced by B. H. Willier)

White Leghorn embryos of 10 to 14 days of incubation have been decapitated with 10 to 20% survival to day 20. The egg is supported with pointed end down and a $\frac{3}{4}$ inch opening is made in the shell over the air space. A small area of the

chorio-allantois and inner shell membrane is rendered transparent with paraffin oil. After locating the embryo, its head is brought through a slit in the membranes and allowed to hang over the edge of the shell while the circulation to the head is stopped with two ligatures about $\frac{3}{8}$ inch apart. The head is severed between the ligatures and the embryo is returned to normal position with very little loss of blood. Cellophane and melted paraffin may be used to seal the opening in the shell, and the egg is incubated in the operating position. The entire procedure requires less than 10 minutes, and is perhaps the simplest method for "hypophysectomizing" embryos. Since the body of the embryo below the proximal ligature appears normal, this technique may be employed for studies on the development of the thyroid as well as other endocrine glands in the absence of the pituitary.

213. *The effect of thiourea on male Fundulus heteroclitus.* Harriet A. CHAMBERS, Bingham Oceanographic Laboratory, Yale University. (Introduced by Dorothea Rudnick)

This experiment was conducted from April 11 to May 24, 1950. During the first month, ten *Fundulus* received tri-weekly injections of 10% thiourea, .005 cc. per gram body weight. During the next two weeks, the strength of the thiourea was reduced to 5%. A matched group ("pair-fed controls"), receiving saline injections, were fed approximately the same amount that was accepted by the experimentals. A third group was untreated and fed ad libitum. After one month, with ten surviving pairs, both injected groups lost weight similarly; neither group grew in length. At the termination of the experiment, weight and length calculations for seven surviving experimentals showed that they had lost 10.2% in weight, while the "pair-fed" controls were back to 98% of their original weight. Untreated controls gained 16.9% in weight. Growth in length was 5.1% for untreated controls and 2.4% for "pair-fed" controls, whereas the experimental fish showed no significant change. Livers of thiourea treated fish were relatively large, gonads regressed. Hepatosomatic indices: untreated controls 3.2%, "pair-fed" controls 2.3% and experimentals 4.0%. Respective gonosomatic indices: 4.0%, 5.0% and 0.64%. Similar effects are produced by hypophysectomy (Pickford, 1950) and may, accordingly, be the result of thyroid inactivation.

For comparison, a sampling of wild population males gave the following hepatosomatic and gonosomatic indices: November, 6.8%, 0.7%; May, 2.8%, 5.6%.

214. *The semilunar cartilages in the knee joint of the fetus.* Simon B. CHANDLER, Department of Anatomy, West Virginia University Medical School.

Observations on the semilunar cartilages in 25 fetuses (11 male, 14 females) show:

1. The general shape of these cartilages is similar to a capital "C" or a small "o". The lateral cartilage was like a "C" 21 times and "o" shaped 29 times. The medial cartilage was like a "C" 35 times and "o" shaped 15 times.

2. The two cartilages are seldom alike. The size varies considerably. In some specimens they cover most of the articular surface of the tibia and other instances they occupy an area along the periphery. Both dorsal and ventral horns attach to the margins of the tibia.

3. A well defined transverse ligament was not observed.

215. *Glycogen in the embryonic and adult uropygial gland.* Sidney A. COHN, Biology Department, Brown University. (Introduced by William Montagna)

In chick embryos, the uropygial gland develops as a double invagination of the epidermis on the 10th to 11th day of incubation. On the 11th to 12th day, proliferation of epithelial cords from the primary diverticulum forms the buds of the presumptive glandular tubules. Growth of these cords and the subsequent development of adventitial buds establishes the definitive gland. On the 16th to 17th day, the cells of the original diverticulum and its lumen become laden with lipid droplets, indicating that the gland has become active. The adventitial buds show little lipid at this time. Glycogen, demonstrated by the periodic acid-Schiff (P.A.S.) technique of McManus, is observed in the epithelium of the early diverticulum when it is first formed. It is present principally in the luminal surface and in the basal cells of the epithelium. The cells of the growing buds of presumptive tubules also contain increasing quantities of glycogen as incubation progresses. From the 17th day to the time of hatching, glycogen is primarily concentrated in the distal extensions of the adventitial buds. In the adult gland, glycogen is found only in the basal cells of the proximal segments of the secreting tubules, dividing the gland into two regions: a central portion around the lumen in which the tubules contain glycogen, and an outer zone whose tubules are free of glycogen.

216. *On the effects of natural gas on the ovaries of the guinea pig.* C. Kenneth COLLINGS, Departments of Anatomy, Baylor University College of Dentistry, and Baylor Research Institute. (Introduced by John A. Cameron)

Cameron (1949) has reported the effects of natural gas on spermatogenesis in the guinea pig. The present experiments indicate a similar inhibitory effect of natural gas upon follicle formation in the ovaries. Nine experimental animals were exposed simultaneously, at 12 hour intervals, to Dallas city gas for 30 to 40 seconds. One animal was sacrificed each week for six weeks with one unexposed control the first week and one on the sixth week. The remaining three exposed animals were sacrificed at two week intervals to see if recovery occurred. Tissues were fixed in 5% formalin and stained in Delafield's hematoxylin and eosin.

After two weeks, the ovaries showed a reduction in the number of ripening follicles to about one-half the normal. At three weeks the number was reduced to about one-third normal and the count remained at this level throughout the remaining three weeks of exposure. The follicles at this time were uniformly large and in the vesicular stage. The follicles appeared normal except for the size variations expected, with egg cells present, each in a cumulus oophorus. The

zona pellucida and corona radiata were plainly visible. Primary follicles were rarely found after three weeks.

The recovery series is not complete at this writing but early sections indicate return of size variations and increased numbers.

217. *The cerebral cortex of the infant at the age of six months.* J. LeRoy CONEL, The Children's Medical Center.

This study is a continuation of the investigation of the postnatal development of the human cerebral cortex, and the results are described in detail in Volume IV, which will appear early in 1951. The same criteria of microscopical development are used as were employed for the cortex in the earlier stages. According to these criteria growth has occurred in some degree in all areas of the cortex since the three-month stage, but has been especially active in the primary efferent area FA γ and the primary afferent areas PB, OC, and TC, in this order. In each lobe of the cerebral hemisphere activity in development decreases directly with the distance from these primary centers. In all these primary centers growth has been particularly active in the large cells in lamina IIIc. Within area FA γ growth has been more active in the region of the hand than in any of the other functional regions. This is probably correlated with the increased use of the hand by the infant as it advances in age. In all areas of the cortex the extra-large pyramidal cells in layer V are more advanced in development than any other cells: the cells in layers V and VI are more advanced than those in layer III; growth has been least active in layer II.

218. *A thoraco-gastroschisis in the rabbit.* Dorcas D. CRARY, Rosecoe B. Jackson Memorial Laboratory. (Introduced by Meredith N. Runner)

Thoraco-gastroschisis is sufficiently rare that a description of its occurrence seems noteworthy. Of 41,500 animals from the colony now at this Laboratory, only one instance has been noted. The rabbit was an albino of Lurie's C race inbred for 10 generations. Examination showed the liver to be herniated, fused to the diaphragm and multilobed, the right anterior lobe being split into four parts, the other three lobes normal. The two halves of the embryonic sternum had not united but were completely separated with a thin membrane stretched between them. The heart was suspended free and not enclosed in a pericardial sac. The lungs were small and covered by a pleura which probably represented the mediastinum. The diaphragm was attached to the manubrial half on the left side; on the right it was less complete. Although the rabbit was full term, the upper incisors were not erupted. The lower incisors were present, the tongue was somewhat protruding and very large. Both cranioschisis and cleft palate have also occurred in the same race, leading to the suspicion that these embryonal arrests may be expressions of the same genetic control.

219. *The effects of daily injections of cortisone in the golden hamster (Cricetus auratus).* L. V. DOMM and Pierre LEROY, Department of Anatomy and Walter G. Zoller Memorial Dental Clinic, The University of Chicago.

The effects of subcutaneous injections of cortisone (Cortone, Merk) were studied in 2 groups of golden hamsters. The first lot of 1 male and 2 females, age 1 month and averaging 51 + grams, received 3.75 mg. daily for 16 days. The second lot of 10 males and 10 females, age 9 weeks and averaging 71 + grams, received 2.50 mg. daily for 30 days. Animals were weighed on alternate days.

In the first group 1 female died on 13th, the other on 14th day of treatment. The male was killed on the 17th day. In each a large abscess, previously palpable, was found on left mandible and the left eye was infected. In second group 7 males and 8 females died between 16th and 28th day of experiment. At conclusion of experiment 3 male and 2 female survivors were kept under observation; all died during following 8 days.

Treated animals showed loss of weight, more pronounced in females. On post-mortem all injected animals revealed abscesses, very marked in some. Eyes, kidneys, and small intestine were most frequently involved though salivary glands, liver, tongue and feet also showed abscesses. In some kidneys were hemorrhagic and in 5 intestine was perforated as consequence of abscesses. The thymus, spleen and adrenals were reduced. Livers showed no conspicuous hypertrophy even where they revealed foci of abscess. Body fat was evident in all, very abundant in some.

220. *Glycemia as related to neurosecretion from hypothalamic neurons.* Glenn A. DRAGER, University of Texas Medical Branch.

The glandular characteristics of certain nerve cells in the hypothalamus are well established. Drager (1949) reported that hepatectomized animals (reptiles) invariably show a depletion of the neurosecretory substance from the magnocellular portion of the hypothalamus. Pancreatectomy, nephrectomy, and castration do not produce such changes. Thyroidectomy and hypophysectomy result in a slight depletion of the secretory material. Administration of dextrose in massive doses does not alter the normal findings. On the other hand, large doses of insulin (320 to 400 u per kg, administered over a period of 3 to 4 days) depleted the hypothalamus of secretory granules in the snake. It is concluded that hypoglycemia produced by any means will result in a depletion of the secretory granules from the hypothalamic neurons. The seasonal variation of neurosecretion that Scharrer described in certain fishes may possibly be explained on the basis of the seasonal variation in the available food of such forms. The above mentioned conditions indicate an interrelationship between the diencephalic gland and carbohydrate metabolism.

221. *Atrophy of motor root fibers following destruction of sensory roots in the rat.* Mac V. EDDS, Jr., Biology Department, Brown University.

The observation that axons of functionally overloaded neurons hypertrophy has suggested that nerve fibers might atrophy if their cytons were to become chronically hypoactive following the elimination of part of their afferent stimuli

(Edds, J. Comp. Neurol., 1950, 93: 258). To test this possibility, the sensory root of the 4th or 5th lumbar nerve (or both) was resected unilaterally in 5 adult rats, thus interrupting the peripheral sensory inflow normally reaching motor neurons through one or both of these nerves. After intervals ranging from 8 to 154 days the diameters of the medullated fibers in the motor roots of the operated nerves were compared with those of the contralateral normal motor roots. The fiber size spectra, cumulative percentage curves and mean fiber diameters derived from these measurements, show an atrophy of the experimental fibers which is already maximal after 8 days. On the average, few fibers have atrophied through more than a single size class (1μ). The reduction in the diameter of small ($< 6\mu$) fibers is slight and not significant statistically. The mean caliber of fibers in the larger size classes (6 to 15μ) is reduced about 7.7% ($P = .0009$). Statistically significant changes in the experimental cumulative percentage curves demonstrate that of the larger fibers those in the ranges 6 to 9μ and 12 to 14μ have undergone the greatest atrophy. These results provide further evidence that both functional and peripheral factors are involved in determining the size of nerve fibers.

222. *Male hormone effects upon the claws of juvenile box tortoises.* Llewellyn T. EVANS, Department of Reptiles and Amphibians, American Museum of Natural History, N.Y.C.

In *Terrapene c. carolina*, the male is distinguished from the female by its larger, more abruptly curved claws of the first 3 digits, rear feet. In the preliminary courtship, the male mounts the female and hooks these claws upon her plastron which is promptly closed, effectively holding the male's claws in a vice-like clamp (sometimes for two hours by actual observation in the mating season). During the interval that the male is held prisoner on the female's back, he bites at her carapace and nips her neck and head.

Careful measurements reveal that juveniles, $2\frac{1}{2}$ times smaller by weight than adult males and 2 times less than adult females, which had been treated with pellets of testosterone propionate had rear claws that averaged 38% longer and were thicker than those of untreated specimens of the same species and of the same approximate size. In fact, the average length of the first 3 rear claws of treated specimens averaged 6.9 mm. in length; those of untreated adult males 7.6 mm; those of untreated females (adult) 6.2 mm; and those of the same size as treated specimens 4.3 mm. The degree of curvature of the treated claws was similar to that of adult males. Over a year and a half elapsed from the time pellets were administered before noticeable changes in the claws appeared.

223. *Comparative effects of pyridoxine deficiency and inanition on testosterone action in mice.*¹ E. D. GOLDSMITH and R. F. NIGRELLI (with the technical assistance of Leonard Ross), Department of Histology, New York University College of Dentistry, and New York Aquarium, New York Zoological Society.

Four month old mice castrated at 25 to 30 days of age and maintained on a Purina chow ration were divided into 4 groups and fed: (1) a synthetic pyridoxine-deficient diet supplemented with 0.25 mg. desoxy pyridoxine administered

parenterally, daily, (2) a synthetic complete diet, (3) a restricted Purina ration, adjusted so that the daily loss of weight was equal in the animals in groups (1) and (3), (4) Purina chow *ad lib*.

After 50 days, when the mice in groups (1) and (3) had lost about 1/4 of their initial body weight, daily parenteral administration of 1 mg. of testosterone propionate (Ciba) in 0.04 cm³ sesame oil was initiated. All animals were sacrificed 24 hours after the 5th injection. At the time of autopsy the survivors in groups (1) and (3) had sustained a loss of about 1/3 their initial weight. Seminal vesicles and prostates were removed and weighed on a Roller-Smith torsion balance, and prepared for histological examination.

The accessory genitalia in the testosterone-treated animals, both on an absolute and on a mg. per 100 gm. body weight basis were significantly lighter in the pyridoxine deficient and restricted diet mice. In these 2 groups the weight of the sex accessories were approximately the same.

Since previous related studies utilizing folic acid antagonists had yielded similar results, the conclusion that the response of the accessory sex organs in the castrate mouse to a sufficient supply of male sex hormone is governed by an adequate food intake and utilization appears justified.

¹ This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

224. *Accessory spleens: a pilot study of 600 necropsies.* Béla HALPERT and Wayne L. EATON, Laboratory Service, Veterans Administration Hospital and Department of Pathology, Baylor University College of Medicine.

The incidence of accessory spleens was investigated preliminary to a study of lesions occurring in them. The necropsy records of 600 patients in whom special search was made to locate accessory spleens were reviewed. Accessory spleens were encountered and microscopically verified in 62 instances. Their sizes ranged from 0.2 to 3 cm. In 52 instances one accessory spleen was present, in 6 there were two, in 3 there were three, and in 1 instance there were several accessory spleens with absence of a spleen proper. There were 23 among 205 patients in the first decade of life, 3 among 14 in the second, 3 among 37 in the third, 6 among 44 in the fourth, 5 among 51 in the fifth, 10 among 129 in the sixth, 6 among 71 in the seventh and 6 among 49 in the eighth decade of life. The oldest patient having an accessory spleen was 75 years old. It is concluded that the overall incidence of 10% is a reliable estimate of the occurrence of accessory spleens.

225. *The distribution of esterases in nerve tissue of the dog.*¹ Walter L. HARD and Merle D. FOX, Department of Anatomy, School of Medicine, University of South Dakota.

Tissue from various parts of the neuraxis have been treated by the method of Nachlas and Seligman ('49) which purports to show sites of "non-specific" esterase enzyme activity.

The most intense reactions were found to be associated with motor nuclei, typical of which is the anterior horn. In this site the motor cells are very

reactive with the processes sharply delimited by virtue of the enzyme reaction. In addition, an intense reaction occurs in the neuropil which cannot be attributed entirely to nerve fibers since glial cells are positive. Motor nuclei such as the oculomotor, vagal, dentate, tectal, accessory, red, and hypoglossal exhibit a reaction similar to that in the anterior column. Basal ganglia, thalamic, and interstitial nuclei are less reactive with the enzyme being confined almost entirely to the cellular elements. Cortical tissue of both cerebrum and cerebellum is persistently negative. Fiber tracts are essentially negative although occasional reactions have been obtained in the dorsal funiculus, cerebellum, and dorsal roots. In the peripheral system, spinal and sympathetic ganglia are intensely reactive both for the cells and the surrounding capsular tissue.

Assuming the reliability of this technic in identifying "non-specific" esterases, these studies suggest this enzyme(s) is subserving an important metabolic function to nerve tissue, and one which is presumably distinct from, and in addition to, that of specific cholinesterase.

¹ This study has been supported by a grant from the U. S. Public Health Service.

226. *Mechanisms of substrate utilization by chick heart fibroblasts in dialyzed media.* Morgan HARRIS, Department of Zoology, Berkeley.

Explants will proliferate for at least three months and probably indefinitely in sugar-free dialyzed media (plasma-serum-embryo extract). Supplemental sugar increases outgrowth while added amino acids inhibit or have no effect. To study mechanisms of utilization, supernatant fluids from cultures, from blanks (cultures incubated without explants), and from stock media were deproteinized and analyzed. Sugar appearing by hydrolysis was detected by osazone formation and depletion of added sugars measured by titration. Lactic acid and amino nitrogen were determined with a Beckman spectrophotometer. Results show that in the presence of explants several hexoses, maltose, and glycogen are depleted with production of lactic acid. Incubated blanks remain sugar-free; added glucose does not form lactic acid and is not depleted by the further inclusion of adenosine triphosphate (ATP). Supplemental maltose and glycogen are hydrolyzed to glucose in blanks as well as in the presence of explants. The level of amino nitrogen in deproteinized supernatants is greatly reduced by dialysis but increases in incubated blanks and in cultures containing explants. Both stock media and supernatant culture fluids show a positive biuret reaction after maximal protein removal with tungstic or trichloroacetic acid. These data suggest that cells are maintained in dialyzed media by the enzymatic breakdown of complex substrates. The enzymes concerned may act externally in the medium as well as in the cells themselves.

227. *The "flat" anomaly of rabbit ovaries.* Berton F. HILL, Jr., Jackson Memorial Laboratory and the University of Chicago, Public Health Service Research Fellow. (Introduced by Paul B. Sawin)

In contrast to the typical, kidney-bean shaped ovary of the rabbit, a high incidence of the "flat" ovary was first reported in a strain of New Zealand Whites being used for Friedman tests by a local hospital. Grossly, these ovaries

exhibit a dorso-ventral flattening, a decrease in width and an antero-posterior lengthening. Microscopically, a large trabeculum of connective tissue runs an antero-posterior course, joining the tunica albuginea at either end. Concurrently, a decrease in the amount of connective tissue of the septa, thecae and tunica albuginea may occur.

Subsequently, this anomaly has been observed to occur at autopsy with varying incidence in most of the races at the Jackson Memorial Laboratory and with a particularly high frequency in inbred race III and its sublines (20%), indicating a genetic influence.

On the basis of internal structure, the ovaries may be classified in groups extending from the rounded "normal" condition to the completely "flat" ovary, thus lending credence to the hypothesis of polygenic control.

Whether these genetic growth processes are mediated by hormone systems, or are the result of known existing regional growth areas, is a matter now under investigation. The fact that the incidence is so great in race III, which is known to have an enlarged lumbar region, is evidence in favor of the latter hypothesis.

228. *A study of the hair structure of mummies found in Utah and Peru.* E. L. HOUSE, Department of Anatomy, New York Medical College.

Each hair was imbedded in paraffin and sectioned at five to ten micra in a plane exactly perpendicular to the axis of the shaft. Some were cleared and mounted unstained while others were stained in various ways. These were then studied in an effort to determine whether the Cliff Dwellers of western United States were related to the people of ancient Peru.

229. *Morphogenetic influence of the spinal cord on the axial skeleton and musculature.* Howard HOLTZER, Department of Zoology, University of Chicago. (Introduced by Paul Weiss)

A formative role of the larval spinal cord in the organization of the vertebral arches is indicated by the following observations: When a four-segment length of sacral spinal cord of the early *Amblystoma* larva is displaced to a more dorsal location, it becomes enveloped by cartilage. Lodged between the longitudinal muscle masses, the cord fragment serves as a core for vertebral arch formation, presumably from migratory skeletogenous cells. The degree of organization of this skeleton varies, depending upon age at operation and damage to myotomes, from well formed segmental arches with outlets for nerves to irregular cartilaginous masses. Preferentially this cartilage lies along the ventral surface of the cord fragment, for when the displaced cord had been rotated by 180 degrees, a conspicuous rod, and in favorable cases even an inverted vertebral arch, may form along its originally ventral surface. The condition of the host notochord in the operated stretch varies from a complete lack of cartilaginous envelopment to chaotic masses of attached cartilage.

If the displaced spinal cord intrudes into the dorsal fin, a supernumerary tail of considerable length is induced. Such a supernumerary tail contains, besides

the displaced spinal cord, segmental muscles, a cartilaginous rod along the originally ventral surface of the spinal cord, ganglionic masses and tail mesenchyme.

¹ Work under the direction of Dr. Paul Weiss aided by the American Cancer Society through the Committee on Growth and the Abbott Memorial Fund of the University of Chicago.

230. *Mitotic activity in the rat hypophysis after removal of the adrenal, thyroid and parathyroid glands.* Thomas E. HUNT, Rufus VAUGHN and Jane CASON, Department of Anatomy, Medical College and School of Dentistry, University of Alabama.

The adrenals or thyroid and parathyroid glands were removed in a series of ovariectomized rats in order to determine whether the mitosis stimulating effect of estrogen on cells of the anterior hypophysis is direct or indirect. An indirect effect seemed possible because increased mitotic activity is not apparent until about 50 hours after injection and does not reach a maximum until about the 65th hour. In both control and experimental groups, ovaries were removed at 60 days. Adrenals and thyroids were removed in the experimental group on the 94th day and four days later all animals were injected with 83 micrograms of estradiol benzoate in oil. Approximately 66 hours after the injection the animals were killed. The average mitotic activity in the hypophyses of the adrenalectomized animals was reduced slightly, but perhaps not significantly, below that of the controls. No significant difference was found in the rats with thyroids and parathyroids removed. It thus appears that the effect of estrogen on the hypophysis is probably direct, causing a release and diminution of secretion which, judging by evidence presented previously, is the factor responsible for mitosis.

- 230a. *Phosphomonoesterase content and localization in the meso- and metanephros, of the chick embryo.* L. C. U. JUNQUEIRA, Department of Histology and Embryology, School of Medicine, University of S. Paulo (Brasil).

The alkaline phosphatase and ribonuclease removable basophilia were investigated histochemically in meso- and metanephros of chick embryos of several ages. In addition chemical determinations of alkaline and acid phosphate content were made. The data obtained suggest that the mesonephric activity begins between the fourth and fifth day of incubation and decreases rapidly between the sixteenth and eighteenth day.

The onset of metanephros function is probably on the eleventh day of incubation.

Our data suggest that the metanephric function is decreased in the last four days of the incubation period. After hatching there is a marked increase in the alkaline phosphatase content.

The ribonuclease removable basophilia can be correlated with differentiation in the meso- and metanephros. Differentiation of the mesonephros is more rapid and uniform than in the metanephros.

231. *The presence of an epithelial membrane in pulmonary alveoli of the mouse at birth.* Vernon E. KRAHL, Department of Anatomy, University of Maryland Medical School.

Shortly before birth the epithelial linings of many of the pulmonary alveoli in the mouse are disrupted by capillaries which rise to the surface of the alveoli to form the so-called "bare areas." Some alveoli become partially expanded by the accumulation of fluid within them. However, a number of them remain completely atelectatic and epithelial membranes lining the alveoli can be demonstrated both in fresh specimens and in fixed and stained sections. Following birth, the transition from the atelectatic to the expanded condition is a very rapid one and the majority of alveoli then have walls which consist chiefly of naked capillaries. However, a study of freshly removed lungs shows that a number of alveoli remain atelectatic for a time and exhibit an epithelial lining.

232. *Virchow-Robin spaces, spaces of His-Held, and their relation to the membrana limitans gliae perivascularis.* Hartwig KUHLENBECK, Department of Anatomy, Woman's Medical College of Pennsylvania.

The perivascular spaces of the brain were studied in pathologic and normal human material.

Virchow-Robin spaces lie between loose, minute adventitial trabeculae of cerebral veins and arteries. A thin outer adventitial membrane is fused with the membrana limitans gliae. In larger vessels, a solid inner layer of adventitia is fused with the media. Outer and inner layers are interconnected by fine strands and blend at the brain surface into the leptomeninges without definite boundaries. In accordance with the original authors, the space of Virchow-Robin should thus be defined as intra-adventitial. This space ends at the transition of arterioles or venules to capillaries and is completely separated from the nervous parenchyma by the membrana limitans gliae.

Along the capillaries, the membrana limitans fuses with the outer surface of the endothelial tube. Membrana limitans and capillary endothelium constitute the blood-brain barrier. Membrana limitans blends with and is reinforced by the astrocyte pedicles.

The space of His-Held represents a locus minoris resistentiae within the nervous parenchyma adjacent to the membrana limitans. It is a potential space which may become a shrinkage artefact or an actual space filled with edema fluid. The periganglionic spaces of Obersteiner are of the same nature as the spaces of His-Held with which they may be continuous.

- 232a. *Ageing of elastic fibers.* A. I. LANSING, Department of Anatomy, Washington University School of Medicine.

The physical appearance and tinctorial properties of human arterial elastic fibers change with age. Elastin extracted from young and old human aortas using 0.1N NaOH-1 (Lowry) shows an increase with age in calcium and phosphorus

contents. Paper chromatography as well as quantitative bioassay for amino acids reveal a sharp increase with age of both aspartic and glutamic acids. These dicarboxylic amino acids may account for the increased base binding capacity of old elastin.

233. *An experimental study of the voluntary motor deficits produced by lesions involving dorsal roots and parietal cortex.* A. M. LASSEK, Department of Anatomy, Boston University.

In a series of 35 monkeys (*Macacus mulatta*), lesions were placed either in the dorsal roots of the brachial plexus or parietal cortex independently or in combinations. Total posterior rhizotomy (C2 to T4) on the left side of mature animals produces voluntary motor deficits more severe and enduring than unilateral removal of the motor cortex. The hands and digits are most affected, movements and grasping being practically abolished (up to four months). In these experiments, the opposite area 4 and the corresponding motor roots remain electrically excitable at low thresholds of stimulus. Very little restitution of function occurs following this operation in a four weeks old monkey (kept seven months to-date). By contrast, the maintenance of just one intact dorsal root (either C5-6-7-8 or T1) of the brachial plexus permits a relatively wide range of motion in mature animals. The degree of impairment is then slight being more of a weakness or clumsiness which is most pronounced when either C5 or T1 are left intact. A total unilateral parietal cortex ablation which alone can produce some motor deficit, when superimposed on the total rhizotomy, does not alter materially the results whereas the opposite sequence enhances it. Bilateral, simultaneously placed lesions involving all parietal cortex cause complete immobilization for as long as animals can be kept alive.

234. *A quantitative study of the endocrine glands of the guinea pig.* Homer B. LATIMER, Department of Anatomy, University of Kansas.

The masses of the four endocrine glands from 110 young adult male guinea pigs were studied to determine the quantitative relationships between them.

The coefficients of variation range from 17.18 for the hypophysis to 29.91 for the thyroid. The testes are less variable than the suprarenals. These coefficients, also those of the percentage weights, are more variable than the coefficient of body weight, which is 12.47.

The correlations between the endocrine glands are all positive but none are significant. The lowest correlation is between the thyroid and the suprarenals, or 0.061 and the highest is 0.355 between the thyroid and the testes. The correlation between the testes and the hypophysis is next to the highest. The averages of each gland with the other glands are: thyroid, 0.161; hypophysis, 0.242; suprarenals, 0.246; and with the testes, 0.317.

Only one of the correlations between these glands and body weight and length, brain weight, eyeballs, and the cord weight and length is negative, namely, that between the suprarenals and brain weight. None of these correlations are significant, except those between the testes and body weight and length, and the cord

weight and length. The average of the correlations between each of these glands and these six dimensions is significant only for the testes, or an average correlation of 0.553.

Regression lines, with empirical formulae have been prepared.

235. *Protection against alloxan diabetes with cobalt, zinc, and ferrous iron.*
Arnold LAZAROW and J. W. PATTERSON, Department of Anatomy,
Western Reserve University.

Various metals were injected intravenously (in amounts just below the lethal dose) immediately preceding (or 15 minutes after) an intravenous diabetogenic dose of alloxan (40 mg/kg or 0.25 mM/kg). The incidence of diabetes at 2, 3, and 7 days was compared to that of a control group of animals receiving alloxan alone. Zinc (.100 mM/kg), cobaltous (.017 mM/kg) or ferrous (.108 mM/kg) ions when injected immediately preceding (but not after) alloxan decreased the incidence of diabetes by 50% or more ($p < .002$). Zinc in half of the above dose only protected 20% of the rats. Manganous (.100 mM/kg), magnesium (.750 mM/kg), cuprous (.045 mM/kg), cupric (.063 mM/kg) and ferric (.100 mM/kg) ions exerted no significant protection.

Sulphydryl groups have previously been shown to protect against alloxan diabetes. Combination (chelation) of the above metals with alloxan may account for their protective action and therefore these results are consistent with the theory that diabetes may also be produced by binding essential metals. However, cobalt was 5 times as effective as zinc or ferrous iron in preventing alloxan diabetes. Furthermore, the diabetogenic dose of alloxan, calculated on a molar basis, is 15 times that of the injected cobalt. Since cobalt is an essential part of vitamin B₁₂, and since vitamin B₁₂ may play a role in reducing disulfide compounds to sulphydryl there is a possibility that sulphydryl and metal protection are interrelated. This is being investigated.

236. *Linear increase in rabbit and rat tendo calcaneus.* E. W. LOWRANCE, Departments of Anatomy, University of South Dakota and University of Missouri. (Introduced by M. D. Overholser)

Two stainless steel markers were inserted near the ends of one tendo calcaneus in each of six 12 to 14 day old rabbits and twenty 26 to 38 day old albino rats. The opposite tendon in each case served as a control. The positions of the steel markers were indicated also by tattoo marks on the tendon. X-Ray pictures of the tendons were made immediately after operation and at weekly intervals thereafter. Serial data thus were obtained for each animal. At the end of the period of study the animals were reoperated, and the positions of the tattoo marks compared with those of the steel markers. The positions were approximately the same, and actual distances between markers corresponded with recorded distances on the final roentgenograms.

During the 58 day growth period beginning 12 to 14 days after birth, the distances between steel markers in rabbit tendons increased 2.7 to 5.7 fold. In the young rabbit the tendo calcaneus appears to increase throughout its length, not merely near its points of attachment. In rats, during the 65 day period beginning

26 to 38 days after birth, distances between markers showed no consistent increases. Linear increase in this tendon in the rat, if similar to that of the rabbit, must have occurred prior to the twenty-sixth postnatal day. Additional studies are in progress.

237. *Maintenance of pregnancy in hypophysectomized rats.* Wm. R. LYONS, Division of Anatomy and Institute of Experimental Biology, University of California.

Hypophysectomy at mid-gestation does not interrupt pregnancy in the rat. To maintain pregnancy until mid-term or later, pituitary gonadotrophins, FSH, ICSH and mammothrophin are effective. In hypophysectomized rats 200 micrograms of an FSH-ICSH combination which caused estrin secretion and 2 mg. of pure mammothrophin which caused progestin secretion by the ovaries maintained pregnancy. Also adequate was treatment with 2 mg. mammothrophin and 1 microgram estrone. After both hypophysectomy and oöphorectomy 4 mg. progesterone and 1 microgram of estrone maintained pregnancy.

The experiment of injecting hypophysectomized rats with the FSH-ICSH combination plus progesterone remained, and was accomplished as follows: Rats received daily subcutaneous hormonal injections from the day of hypophysectomy (1 day after sperm) through day 11 with necropsy on day 12. Group 1 (8 rats) received 200 micrograms FSH-ICSH and 4 mg. progesterone. All showed maintenance of pregnancy. Group 2 (3 rats) received 60 micrograms FSH-ICSH and 4 mg. progesterone. All showed resorption sites. Group 3 (5 rats) received 4 mg. progesterone. Two showed resorption sites and 3 showed no evidence of implantation. Group 4 (4 rats) receiving 60 micrograms FSH-ICSH and Group 5 (4 rats) receiving 200 micrograms of FSH-ICSH showed no evidence of implantation.

238. *Effects of grafted testes upon the reproductive organs of castrated fetal rats.* Edward L. MAXWELL and L. J. WELLS, Department of Zoology and Department of Anatomy, University of Minnesota.

Using the litter as experimental unit, ten fetuses were castrated at about 19 days and 13 hours after observed matings and killed just prior to expected parturition (treatment, about 51 hours). In each of nine other castrated fetuses, four fetal testes were subcutaneously grafted. Nineteen controls were used. After fixation of tissues, a block of each grafted region and a block of each pelvic region were sectioned in series. The volume of the seminal vesicle was determined from the sectional gland and converted into mm.³ per gram of body weight.

Some castrated fetuses showed atrophic changes in the ductus deferens, thus suggesting surgical trauma as one causative factor. All showed a retarded growth of the seminal vesicle. Length of the flexed portion of this gland was about one-third that of the control. That absence of the testes was a second causative factor is suggested by the prevention of all these changes by implanted crystalline androgen (Abstract 23).

The grafted testes failed to prevent these effects of castration. The grafts had become vascularized, especially at the periphery where a shell of testicular tissue

(tubules and interstitial cells) resembled the tissue of control testes. The interstitial cells were fewer than in controls. Centrally the grafts were necrotic.

These observations neither support nor discredit any particular theory regarding the possible role of hormones in sexual differentiation in mammals.

239. *Studies of the peripheral blood of hereditary dwarf mice.* Edwin A. MIRAND and C. M. OSBORN, Department of Zoology, Syracuse University.

Studies including total and differential counts, hemoglobins, and hematocrits were done on 156 samples of blood drawn from the tails of 72 fully grown hereditary hypopituitary dwarf mice (Bar Harbor strain). For comparative purposes similar observations were made on 180 tail blood samples from 120 normal littermates.

The data obtained revealed in general, hemal dilution, erythropenia, leucopenia, and evidence of anemia in the dwarf as compared with blood data of normal mice. Erythrocytes averaged 24% below normal; leucocytes 31.5% below normal; hematocrits were reduced by 16% (cell size not significantly different in dwarfs and normals); and hemoglobin concentration was 15% lower. It is of interest that qualitatively similar hematological observations have been reported in hypophysectomized mammals.

Differential counts revealed neutrophils in dwarfs to be decreased by 38% while the lymphocytes proved to be 13% more numerous than in normals. Eosinophils, basophils and monocytes were not significantly different in normal and dwarf mice.

Physiological studies on the blood relating endocrine abnormalities and carbohydrate metabolism are in progress.

240. *Further observations on the selective stoppage of bone growth in tissue culture.* George H. PAFF and Francis C. EKSTEROWICZ, Division of Anatomy, Hahnemann Medical College.

Recently we used alizarin Red S to stop selectively the growth of bone tissue. This work has been extended to a study of fibulas in which no osteogenetic activity is evident. Will alizarin prevent the appearance of osteogenetic activity?

After extensive exploratory work fibulas of 8-day chick embryos lacking an alizarin receptive zone were selected. This zone is a region of mineralization which invariably precedes the formation of spicules. Spicules never form anywhere but within it. It is the first stage in osteogenesis.

Routine hanging drop preparations were made of 13 pairs of fibulas. One member of each pair was placed on alizarin-free media as a control; its mate on media containing alizarin (concentration of 1 drop of a Seitz filtered saturated watery solution to 120 drops of media). After five days, 1 drop of alizarin ($2\times$'s the above strength) added to each. The following results were obtained:

1. The controls developed an alizarin receptive zone averaging 0.72 mm. in length. Their average total length increase was 0.81 mm. Connective tissue grew luxuriantly.

2. The fibulas growing on alizarin did not develop an alizarin receptive zone. Average total length increase was 0.79 mm. Connective tissue growth was uninhibited.

These results offer additional evidence that alizarin might be useful in controlling undesirable over-production of bone in vivo.

241. *Connections of zona incerta, and the tractus tectopontinus in brain series of horse, sheep and other forms.* James W. PAPEZ, Zoology Department, Cornell University.

Two large fiber tracts enter the zona incerta located in front of the red nucleus. The dorsal supraoptic decussation comes from the lower tegmental region near medial longitudinal fasciculus, passes over the red nucleus to enter the zona incerta where its fibers decussate over optic chiasma and pass laterally to outer division of the globus pallidus or beyond. From the pars ventralis of the lateral geniculate (not from pregeniculate) comes the large geniculoincertain fasciculus which also terminates in zona incerta. Zona incerta sends fibers laterally to outer globus pallidus. Zona incerta may be a subcortical correlation center for optic and vestibular impulses relayed to pallidum.

A large tectopontine tract is prominent in series of brains of horse, sheep, dog, cat and rabbit. It extends from tectum ventrolaterally in front of inferior colliculus and lateral lemniscus. Its medial fibers enter lateral part of isthmie tegmentum, while its lateral fibers form a superficial stratum in isthmie region and enter lateral nuclear masses of pons. The tectopontine tract indicates that a strong tectopontocerebellar pathway is present. Marchi method demonstrates its polarity in cat and rat. The parabigeminal body is found in the course of this tract, but seems to have other connections. The name stratum lemnisci commonly used for tectopontine tract is not appropriate since the bulk of this tract does not come from the lemniscal system.

242. *Oxygen consumption and serum protein-bound iodine levels in the pregnant guinea pig.*¹ Roy R. PETERSON, Mina MacNair BROWN and William C. YOUNG, Department of Anatomy, University of Kansas.

The following groups of animals were established: 10 non-pregnant females 24 hours after heat, 5 to 9 animals each at 20, 30, 40, 50, and 60 days of pregnancy, and 5 females 48 hours after parturition. Determinations of oxygen consumption and serum protein-bound iodine (PBI) levels were made on each animal.

Oxygen consumption in the non-pregnant group averaged 51.8 cc/100 gm/hr. (46.8 to 54.8). In the other animals the values were: 54.0 (42.8 to 60.1) at 20 days; 50.7 (47.1 to 53.7) at 30 days; 50.0 (42.8 to 54.9) at 40 days; 54.9 (49.4 to 61.0) at 50 days; 57.7 (51.2 to 63.1) at 60 days; and 70.8 (64.3 to 81.7) by 48 hours after parturition.

The PBI levels were: 0.89 γ % (0.1 to 1.4) in the non-pregnant group; 0.72 (0.7 to 0.8) at 20 days; 0.86 (0.6 to 1.2) at 30 days; 0.80 (0.6 to 1.1) at 40 days; 0.62 (0.4 to 0.8) at 50 days; 0.72 (0.4 to 1.2) at 60 days; and 0.64 (0.4 to 1.0) by 48 hours after parturition.

The PBI levels neither show the rise early in pregnancy reported by Peters, Man, and Heinmann (1948) for the human female, nor do they reflect the increase in oxygen consumption occurring late in pregnancy and following parturition.

¹ Aided by grants from the U. S. Public Health Service.

243. *The response of hypophysectomized male Fundulus heteroclitus to injections of purified mammalian growth hormone.* Grace E. PICKFORD, Bingham Oceanographic Laboratory, Yale University.

A new experiment confirms the fact that hypophysectomized *Fundulus* do not grow. Fifteen weeks after the operation 20 hypox fish were divided into 3 groups: (1) receiving triweekly injections of growth hormone (A. E. Wilhelmi), 10 $\mu\text{g/g}$ wt, for 9 weeks, (2) receiving saline, and (3) untreated. Length and weight changes were recorded at weekly intervals. Groups (2) and (3) showed no change in length and no new growth on scales or otoliths; there was a progressive weight loss, significantly greater in group (2). A similar trend was reversed at the end of the third week in fish receiving growth hormone. After 9 weeks these fish had increased 14.8% in weight and 4.6% in length. Length increase did not become statistically significant until the end of the sixth week, but 5 out of the 7 fish showed a resumption of growth on scales sampled after 1 month. After 9 weeks the scales of all fish showed new growth, averaging 3-4 new circuli, and all but one showed new growth on the otolith. Resumption of scale growth is accompanied by the formation of a zone of irregular pattern resembling, in an exaggerated degree, the "year mark" laid down on the scales of unoperated controls.

244. *Liver histology of testosterone propionate and methionine treated normal and hyperthyroid mice.* D. R. REISFIELD and J. H. LEATHEM. Bureau of Biological Research and Department of Zoology, Rutgers University.

The influence of hyperthyroidism alone and in combination with testosterone propionate or methionine was studied in 22 day old female mice. Feeding thyroglobulin (0.1%) for 17 days did not alter liver weight whereas 0.1 mg. of testosterone propionate (perandren, Ciba) daily alone or with thyroglobulin as well as the addition of 2% methionine to the diet increased actual but not relative liver weight. 5% methionine reduced liver weight. A marked decrease in alkaline phosphatase was obtained by adding 2% methionine to the diet and some reduction in enzyme was observed following thyroglobulin and testosterone propionate. Liver cells lacked neutral fat, phospholipids, unsaturated glycerides, cholesterol and ferric iron with the exception of liver sections from 2 to 10 mice receiving thyroglobulin and testosterone propionate in which clusters of cells containing fat were seen. Hepatic glycogen was observed throughout the liver lobule in 9 of 11 control mice and in an equal number of methionine fed or androgen treated mice. Hyperthyroidism reduced glycogen so that only 2 of 21 mouse livers exhibited stainable glycogen. However, methionine or testosterone propionate partially protected against the loss of liver glycogen induced by hyperthyroidism.

245. *Azurophilic inclusions in connective tissue cells, produced by suspending agents.*¹ G. L. SCHNEEBELI, S. LOEWEN and T. F. DOUGHERTY, Departments of Anatomy and Pharmacology, University of Utah College of Medicine.

Investigation of the azurophilic fibroblast inclusions previously produced by 1 mg. Cortone acetate (Merck) indicates that they appear within 4 hours and the number of cells affected (40%) is maximal 24 hours after injection. In subsequent weeks, all features of the phenomenon fade slowly.

When the constituents of the cortisone-free suspending agent were studied, sodium carboxymethylcellulose turned out to be chiefly responsible, Tween 80 being also, but only weakly effective. The inclusions appear in fibroblasts and endothelial cells. The phenomenon is strictly topical but simultaneously given cortisone causes affected fibroblasts also to appear ubiquitously in distant connective tissue areas. Tween 80 and, to a lesser degree, carbomethoxycellulose, are phlogogenous and the attracted macrophages are similarly affected as the fibroblasts, but the presence of cortisone prevents their participation. Of other colloids studied comparatively (pectin, polyvinyl alcohol and methylcellulose), only pectin produced azurophilic inclusions.

Daily repeated injection greatly increased the number of affected cells and of inclusions per cell, and the persistence of the phenomenon.

¹ Supported in part by a grant from the Life Insurance Medical Research Fund.

246. *Impaired tolerance to silver in vitamin E deficient rats.* Samuel L. SHAVER and Karl E. MASON, Department of Anatomy, University of Rochester School of Medicine and Dentistry.

Drinking water containing 0.15% silver nitrate was given to rats reared from 21 days of age on a vitamin E deficient diet (23 rats), the same diet supplemented with tocopherols (10 rats), and "Rockland" stock diet (16 rats). The last two groups survived and were sacrificed at intervals up to 18 months of age. Rats on low-E diet, though consuming less water and silver than those on stock diet, showed symptoms of toxicity and muscle dystrophy and succumbed within 18 to 40 days, except for one rat which survived for 7 months. When started on low-E diet and silver nitrate at 28 days of age, 2 out of 6 rats survived this critical period; priming with tocopherols prior to the 28th day enabled 6 others to live for long periods. Silver lactate in the drinking water and silver chloride in the food, providing comparable levels of silver intake, gave similar results.

Histologic findings confirmed those of Gatz (Anat. Rec., 1940, 103, 454) regarding silver deposition in macrophages and glomerular endothelium and in some reticular fibers of lymph nodes and white pulp of the spleen. Silver was deposited also in basement membranes, especially those of the thyroid follicles, salivary gland ducts, proximal convoluted tubules, urinary bladder, and in endothelial cells and often in the media of blood vessels of all calibers.

247. *Preliminary report of electron microscope studies on structure of visual cells of Rana pipiens.* Jerome K. SHERMAN, Zoology Department, State University of Iowa. (Introduced by H. W. Beams)

Eyes of the 8, 9, 10, 11, 12 and 15 mm. tadpole as well as those of the adult frog were fixed in 10% neutral formalin and sectioned at .2 micron according to a modification of the technique of Beams and assoc. (1950).

Electron micrographs indicated the following in sagittal sections of the eye:

The inner and outer members of the visual cell are derived from the cytoplasm. In the 8 mm. tadpole the outgrowth consists of from one to twenty vacuoles in a heterogeneous fibrous network. At 9 mm. a distinct secondary structure appears as an extension of the outgrowth. This new element develops a cross striated appearance and by the 15 mm. stage distinct thick fibers are arranged perpendicular to the long axis of what will be the outer member of a rod, along with a slight hint of longitudinal fibers. Approaching the adult stage, the inner member becomes denser, more homogeneous and loses its vacuoles while the outer member of the rod exhibits, in addition to cross striations, clearly defined longitudinal fibers, the terminations of which were undetected. The inner member of the adult cone retains one large vacuole and its outer member is only cross striated.

248. *A ganglion in the terminal complex of porpoise, rabbit, and man.* John G. SINCLAIR, Department of Anatomy, University of Texas Medical Branch.

Embedded in the inner face of the dura at the junction of the cribriform plate and the frontal bone there is a pair of ganglia close to the midline. These, in the adult porpoise, are branched stellate masses which are not symmetrical. One, with a central mass of about 3×5 mm, contains more than 4780 ganglion cells of two sizes. The other, 1.2×6 mm., contains more than 3592 cells. Long thick branches containing scattered cells extend along blood vessels mainly upward and downward. A few small twigs reach laterally. One ventral branch from each passes down and through the suture region toward the blowhole. This pair of ganglia has been found also in two porpoise fetuses. In these the cells were much smaller than the cranial or spinal ganglion cells in the same animals. Similar masses have been located in the rabbit at birth on the course of the terminal nerve at the same suture line. Fiber connections have not been determined with certainty. In a four-months human infant a pair have been seen grossly, again at the predicted spot, and have been confirmed microscopically. Functional connections are under study in silvered preparations.

249. *Histories of inert and active foreign substances implanted in tadpoles.* Carl Caskey SPEIDEL, School of Anatomy, University of Virginia.¹

As reported in earlier studies, inert substances such as fine strands of silk, nylon, and tantalum, when implanted in tadpoles, are readily walled off and tolerated by the adjacent tissues. Essentially similar results have now been obtained with implants of silica, human hair, cotton, polyethylene, and tygon.

Magnesium, however, when implanted in the tadpole, undergoes progressive disintegration. This metal reacts with water or with body fluid giving rise to mag-

nesium hydroxide and hydrogen. Although leukocytes quickly surround the implanted material, they do not stop the chemical transformation of the magnesium. Typical case histories of individual implants reveal the production and growth of vesicles filled with fluid. In some instances this is conspicuous within a few days; in others it may not be noticeable until much later. In one case a sizable vesicle first developed after 80 days. Presumably the irritant which induces this reaction is magnesium hydroxide. Alternating increase and decrease in the volume of the vesicle may take place from time to time. Pressure injury of adjacent structures may ensue.

Thus, magnesium is a material that causes prolonged and chronic irritation of tissues. This is of surgical interest in the case of war wounds caused by magnesium fragment. Such wounds fail to heal readily (cf. demonstration no. DM3).

¹ Aided by a grant from the American Cancer Society (Committee on Growth).

250 *The spinal border cells of Cooper and Sherrington.* James M. SPRAGUE, Department of Anatomy, School of Medicine, University of Pennsylvania.

Counts of the border cells, chromatolyzed by contralateral lower thoracic hemisection in 2 macaques, totaled 1310 and 1345 on one side of L1-L6; very few cells were found in L7. About 50% of the total number were found in L3 and L4, with a maximum in L4. Comparison with counts of motor cells made by Bodian ('48) in L4-S1 of 4 macaques, showed that the Cooper-Sherrington cells form about 19% of the somatic motor cells (exclusive of the medial nuclei) in L4, 11.5% in L5, and 2% in L6.

Cooper-Sherrington cells are morphologically indistinguishable from somatic motor cells and large propriospinal cells; they are markedly different from those of the proved spinocerebellar relay nucleus of Clarke. The axons of the Cooper-Sherrington cells ascend in the contralateral lateral funiculus and terminate in part in the upper levels of the cord (Morin and O'Leary '50), partly in the cerebellum (Sprague, '50), and probably also in the lateral funiculus of cats, following tactile and proprioceptive stimulation of the contralateral hind leg and tail (Sprague '51). The concept here presented holds that the Cooper-Sherrington system is primarily unrelated to the periphery, but forms an ascending limb in the close relationship between the cerebellum and the reticular and propriospinal nuclei of medulla and spinal cord.

251. *Demonstration of spatial and temporal patterns of reducing enzyme systems in early chick blastoderms by neotetrazolium chloride, potassium tellurite and methylene blue.*¹ Nelson T. SPRATT, Jr., Department of Zoology, University of Minnesota.

It has been suggested (Spratt '50) that the pattern of differential inhibition of development produced by inhibitors or other environmental modifications (starvation, anoxia) coincides with a pattern of underlying enzymatic activity. In 20-30 hr. embryos explanted to albumen or Ringer-glucose media containing the reducing enzyme indicators, neotetrazolium chloride or potassium tellurite, the

(aerobic) spatial and temporal pattern of reduction of the colorless reagents to formazan (red or purple) and tellurium (black), respectively, coincides with the pattern of inhibition and/or developmental activity. The (anaerobic) pattern of reduction of methylene blue is essentially the same.

Regions of embryos where differentiation activities are greater (e.g., the node and head-fold in head-process blastoderms; the node, segmental plate and fore-brain in early somite blastoderms; and the tail-bud in older embryos) have a much more rapid and extensive capacity for reducing the reagents used than other regions. Structures or parts (e.g., somites, chorda, neural tube) which develop during exposure of the blastoderm to non-toxic concentrations of neotetrazolium become pink or red in contrast to the almost colorless appearance of structures or parts formed prior to explantation.

Heat-killed and formalin-fixed embryos exhibit no reduction activity. Starved (pretreated) embryos (4-16 hrs. incubation without substrate) show very weak or no dehydrogenase activity in the continued absence of substrate, but pronounced activity when glucose is present in the indicator medium.

¹ Supported in part by grants from the National Institutes of Health, U. S. Public Health Service and the Graduate School, University of Minnesota.

252. *Numbers of white blood cells in adrenalectomized mice.* Kathryn F. STEIN and Cynthia MARTIN, Department of Zoology, Mount Holyoke College.¹

Thirty normal adult mice of mixed ancestry, proven to be completely adrenalectomized by the epinephrine test (Speirs and Meyer), had higher white counts after operation. Total and differential counts were made and averaged for 10 or 11 individuals at each of the following intervals: 1-2, 4-5, 6, 10, and 17-30 days after adrenalectomy. Total white cells and absolute numbers of mononuclears increased progressively with increase in length of the interval. Percentage increases in mononuclear cells, obtained by comparison of averages from the same group of mice before and after adrenalectomy, were 10%, 25%, 90%, 75% and 100% at the 1-2, 4-5, 6, 10, and 17-30 day intervals, respectively. Percentage increase in eosinophiles was present at 6 days and thereafter and varied from 70 to 100%. Individual mice also showed increases in total count and in lymphocytes and eosinophiles in practically all cases at the later intervals. Comparison of the average numbers of neutrophils for the same animals before and after operation showed increase at some intervals, decrease at others. The increase at 17-30 days was only 10% and was considered insignificant.

¹ Supported by grant from Committee on Research in Endocrinology, National Research Council.

253. *An intensified protargol method for the staining of sections of nervous tissue.* W. A. STOTLER, Department of Anatomy, University of Oregon Medical School.

The essential elements of this method are the use of a dilute protargol solution for the initial impregnation and the simultaneous reduction and intensification of the resultant silver deposit. The old protargol series are more successful. Protargol-S or an admixture can be used. Fix in formalin, Bouins, or acetone.

Sections are impregnated for 18–24 hours in 0.1% aqueous solution of protargol. Place 5–10 grams of copper in the staining dish and dust 0.25 grams of protargol on the water (250 cc) containing the sections. The addition of 4 drops of pyridine and 0.25 grams of sodium glycerophosphate apparently improves the stain.

Place sections without washing on a staining rack and flood with a few drops of the reducing solution. Follow the process of reduction and intensification under the microscope and when optimum, wash, dehydrate, and mount.

Prepare the unstable reducing solution immediately before use by combining the following solutions in order: Solution A, 6% silver nitrate, 10 cc.; Solution B, 20 grams sodium sulfite in 330 cc. water, 10 cc.; Solution C, 35 grams sodium thiosulfate in 330 cc. water, 10 cc.; Solution D, 5 grams sodium sulfite, 8 grams Kodak elon in 1000 cc. water, 30 cc.

The stain is excellent for synaptic terminals in the central and peripheral nervous system, and the pineal and pituitary glands.

254. *The rate of conduction in stretched and unstretched nerve.* R. S. TURNER, Department of Anatomy, Stanford University.

From theoretical considerations it might be predicted that as a nerve is stretched the conduction rate (in meters per second) (1) might be expected to *decrease* as the diameter of the fibers decreased, or (2) might appear to *increase*, if the nerve fibers merely uncoiled as the nerve lengthened. In a mixed nerve the larger fibers might be constricted as the nerve was stretched, thus leaving conduction to the smaller, slower fibers. A large series of measurements has been made on the conduction rates of the fastest fibers in the pedal nerves of *Ariolimax columbianus* under conditions of stretch and relaxation. The results consistently show that such nerves may be stretched to their limits (more than twice their original length) without any alteration in the rate of conduction.

255. *Structure of the cortical areas after hemidecortication in the albino rat brain.* A. VAZ FERREIRA and C. M. COMBS, Department of Anatomy, Northwestern University Medical School.

Eight albino rats were hemidecorticated and allowed to survive 18–60 days, that is, a time sufficient to allow complete Wallerian degeneration of the nerve fibers originating in the destroyed cortex. After silver nitrate impregnation with Cajal's formula 6, it was possible to recognize, as having no obvious modifications, all the cortical areas in the unoperated side. Therefore, it is seen that the interhemispheric fiber connections are not critical in the formation of the characteristic patterns of the cortical areas.

These results were confirmed by the study of five hemidecorticated rats allowed to survive 41–94 days and stained with the Weil myelin technique. The rapid disappearance of degenerative particles stainable with the Cajal formula serves as a definite advantage over the Weil technique wherein stainable particles are known to persist for relatively long periods after injury.

256. *Rapid reestablishment of the cranial vault after its excision in young rats.*

Donald G. WALKER, Institute of Experimental Biology and Division of Anatomy, University of California, Berkeley. (Introduced by Herbert M. Evans)

Regeneration of membrane bone was studied following removal of the roof of the calvarium. In twelve normal female rats, forty-five days of age, the central portion or roof of the calvarium was excised with periosteum, dura mater and associated sinuses.

Three rats were sacrificed on each of five post-operative days. Each preparation was studied under the binocular microscope at the time of autopsy and again after impregnation with AgNO_3 .

On the third post-operative day, a dense membrane was found at the site of the defect. This was attached superficially to the overlying galea aponeurotica and at its periphery became continuous with the undisturbed periosteum and dura. Beneath this membrane vessels had reestablished the superior sagittal sinus and tributaries.

On the fifth post-operative day, the first evidence of intramembranous ossification appeared as bilateral lines of calcification each parallel to but separated from the cut edge of the parietal bone. Two days later these lines of calcification had become broader and longer. At this time a third line had developed in the membrane adjacent to the cut margin of the frontal bone.

By the tenth day each calcified portion of the membrane had united with the bone of the adjacent margin.

By the twentieth day, the membrane was entirely calcified except at the sutures.

257. *Mechanical separation and displacement of the paired primordia of the glycogen body of the chick spinal cord.* Ray L. WATTERSON, Biological Sciences, Northwestern University.

In an attempt to prevent fusion of the paired primordia of the glycogen body and to produce twin glycogen bodies, a glass needle was inserted into the neural canal of the lumbosacral level of the neural tube of 72-hour chick embryos and was lifted in the midsagittal plane, slitting the roof plate and overlying epidermis lengthwise. Thin sheets of celloidin were inserted vertically into these incisions to delay healing. Embryos were killed after 16 days of incubation. Neural arches failed to unite in the operated region and variable amounts of spinal cord protruded dorsally. A neural canal was present although frequently malformed and displaced. The glycogen body was displaced to the surface and was never covered by epidermis. In some cases it lay across the midline on the dorsal surface of exposed nervous tissue. Occasionally it was displaced laterad and lay in large part above the skin; in such cases feather germs were embedded within its substance. Occasionally twin glycogen bodies had formed, one above the skin to each side of exposed nervous tissue. The results can be accounted for by assuming: (1) presence of primordial cells within the roof plate at time of operation; (2) paired distribution of these cells; (3) variations in degree of displacement of lateral halves of the roof plate dorsad and laterad; (4) variations in the plane of incision (midsagittal or parasagittal).

258. *Adrenal cortical changes in the Golden Hamster following a single injection of diethylstilbestrol.*¹ Bernard C. WEXLER, Department of Anatomy, Stanford University. (Introduced by Hadley Kirkman)

A single intraperitoneal injection of 0.06 mg stilbestrol per animal was given to groups of male and female hamsters and the animals sacrificed 0, 1/3, 1, 3, 6, 12, 24, 36 and 96 hours later. Results of chemical analysis of the adrenal glands for ascorbic acid content indicated a marked release of ACTH in female hamsters but a relatively slight release in males following stilbestrol injections. In addition to this marked sex difference in the release of ACTH, histochemical tests showed that the hamster differs from other animals in the manner in which it resynthesizes ascorbic acid. Additional histochemical and histological tests showed no changes in lipid constituents. Though the Schultz cholesterol test was negative in the reportedly "lipid-poor" hamster adrenal, it has been observed in this laboratory that with prolonged treatment with stilbestrol the adrenal becomes markedly Schultz positive in this species. The lipid-free zone in the hamster was found to be broader in the female than in the male. Adrenal weights of both sexes underwent marked changes during the 96-hour course of the experiment.

¹This experiment was supported by grants from the American Cancer Society and the National Cancer Institute. The diethylstilbestrol used was supplied through the courtesy of Dr. D. C. Hines of the Eli Lilly Company.

259. *A study of the relative effectiveness of certain adrenal cortical hormones.* Wayne L. WHITAKER, Department of Anatomy, University of Michigan.

Inhibition in the growth of hair in Long-Evans rats was used as an indication of the relative effectiveness of six adrenal-cortical hormones. Various concentrations of these hormones in absolute alcohol were applied at the rate of 0.1 ml per day to the right dorso-lateral aspect of the neck of the experimental animal. Recorded observations of the promptness of appearance of the inhibition, its completeness and persistence furnished the basis for the comparison of effectiveness.

As an inhibitor of hair growth, 17-hydroxycorticosterone (Compound F) is about 50% more potent than 17-hydroxy-11-dehydrocorticosterone (Compound E, cortisone). Corticosterone and 11-dehydrocorticosterone appear about 40% as effective as cortisone. Desoxycorticosterone and its acetate seem to be somewhat less effective than the corticosterone in this regard.

260. *The staining of the intercellular bridges of the stratified squamous epithelium of the oral and vaginal mucosa by sudan black B and Baker's hematein method.* George B. WISLOCKI, Department of Anatomy, Harvard Medical School.

In the stratum germinativum of the vaginal and gingival stratified epithelium (human, rhesus monkey), the intercellular bridges and especially the desmosomes, or bridge corpuscles, are stained very sharply by sudan black B and by Baker's acid hematein method. The most intense differentiation of these structures occurs in the outer two-thirds of the stratum germinativum involving the stratum spinosum. The bridges diminish in distinctness and vanish progressively in the cells of the cornified outer layers. With Baker's method the staining is not so constant

or uniform as with sudan black. Sudanophilia of the intercellular bridges of human vaginal epithelium was noted previously (Wislocki, Bunting and Dempsey, 1950, *Menstruation and Its Disorders*, published by Charles C. Thomas Company).

The observations with sudan black indicate that the bridges and desmosomes are lipoidal in nature, whereas the positive Baker reaction suggests a phospholipid component. The observations support the concept that the structures are integral parts of the cells and not, as some have maintained, either artefacts or fibrous interstitial material of the nature of cement substance. In poorly fixed epithelia, granular sudanophilic debris occurs in place of the sharply defined intercellular bridges and desmosomes.

261. *Observations on aortic paraganglia (Zuckerland's bodies) in the albino rat.*

Ozro B. WISWELL, Department of Anatomy, University of Texas Dental Branch. (Introduced by Ira R. Telford)

In the albino rat these paired paraganglia are located on either side of the aorta at about the level of its bifurcation. They are well vasculated, glistening, spindle shaped, pearly white masses adjacent to the sympathetic nervous system. Observation of paraganglia length, color, and topography in normal and pregnant animals of a known age and weight are the basis of this report. Forty animals of both sexes ranging in age from 1 to 78 weeks were studied.

On the basis of animal age or weight compared to paraganglia length, it was observed that animals of 1 week of age possessed paraganglia 1.5 mm. long while maximum paraganglia length of 12 mm. was noted at 24 weeks of age or when the animal weighed approximately 240 gms. From this maximum size the paraganglia decreased to an average length of 7 mm. at 80 weeks or an average animal body weight of 335 gms.

Young pregnant females possessed paraganglia similar to much older animals. After parturition the paraganglia were observed to be lobulated and larger, returning in about 8 weeks to normal size.

After recovering from extirpation of the paraganglia both males and females became vicious, difficult to handle, and displayed "nesting characteristics." Young animals became inactive. When sacrificed the heart, kidneys, and intestines were embedded in an excess of fat. After parturition females continued to show swollen mammae.

262. *Hormonal modification of sex differentiation in the obstetric toad, Alytes obstetricans.*¹ Emil WITSCHI, Department of Zoology, The State University of Iowa.

Taxonomists consider *Alytes* a primitive member of the salientia; however, it has a relatively highly specialized urogenital apparatus, with almost complete separation of urinary and genital organs in the male. The male possesses large seminal vesicles, which probably fulfill an important role in the practice of "dry fertilization" which is peculiar to this species. Larval sex differentiation follows a similar course as in frogs, but the maturation phase of spermatogenesis begins relatively early and sperms are present at the time of metamorphosis.

As in frogs, treatment of larvae with estradiol results in partial or complete feminization of the genetical males; but quite to the contrary, the administration of methyltestosterone remains without effect on the development of the ovaries of genetical females. Analysis of the observed changes and comparison with the facts known from experiments with other species lead to the following two conclusions: (1) Steroid hormones, if effective, are inhibitors of either the cortical or the medullary inductor system of sex differentiation. (2) These inductors show varying susceptibilities to hormonal inhibition according to species.

Transitional stages of sex reversal under the influence of moderate doses of estradiol furnish interesting material on medullary oocyte and intersexual germ cell formation, which permits a closer definition of the inductor concept.

¹ Supported by grants from the National Research Council, Committee for Research in Problems of Sex.

263. *Ciliary "rootlets" observed with the electron microscope and with phase-contrast equipment.* Leonard G. WORLEY, Department of Biology, Brooklyn College.

Since the ciliary "rootlets" of ciliated epithelial cells cannot ordinarily be observed in the unfixed cell, the question arises as to whether these actually exist or are merely manifestations of fixative effects on a peculiar type of cytoplasm. Studies of living cells of the oral cavity of the frog in phase-contrast do not demonstrate such fibrils there, but they are revealed with great clarity in the ciliated cells of molluscs. In the latter, the fibrils together form a cone-shaped structure (ciliary "cone") which ends in a single terminal fibril usually well below and entirely avoiding, the nucleus. The ease with which the cilia may be detached from this fiber system belittles its value as a possible system of anchorage "rootlets" for the cilia, as has sometimes been suggested. The ciliary "cone" can be bodily removed from the cell as a discrete fibrous structure. Frequently the terminal fibril is seen poking through the lateral cell membrane as a sharp, stiff filament in bent or twisted cells. Electron photomicrographs have been obtained showing the delicate interconnections between fibrils. In molluscan gill cells, the fibrils appear as a series of plates that narrow to fibril proportions at points of attachment between themselves. Some basal corpuscles in electron photomicrographs appear homogeneous; others present a less dense, or less absorptive, central core.

264. *A study of rats subjected to the environment of a jet engine.*¹ E. H. YEAKEL and R. H. GRIFFEN, Division of Anatomy, Hahnemann Medical College, and the Aeronautical Medical Equipment Laboratory, U. S. Naval Base.

This work is part of a research program of the Aeronautical Medical Equipment Laboratory of the Philadelphia Naval Base. It was undertaken to investigate the possible effects upon animals of exposure to a jet engine. Thirty-two rats of both sexes were divided into the following groups: (1) experimental animals, periodically transported to a test cell that housed a jet engine, there

exposed during a run of the engine, and then returned to the animal colony; (2) rats moved to and from the test cell, but not exposed during the run; and (3) controls that were not moved from the colony. The first two groups were subjected to the above procedure intermittently. All three groups were observed for a year. Increments of body weight followed weekly were found to be slightly retarded in the experimental males, but augmented in the females. During the last three months of the experiment, systolic blood pressures, obtained with a tail plethysmograph while the rats were in the animal colony, showed no changes from normal. Subsequently all of the animals were dissected. All organs appeared to be normal grossly. Heart, liver, kidneys, adrenals and thymus were weighed; their absolute and relative weights in experimental rats were not significantly different from control values. It is concluded that neither gross anatomical alterations nor permanent changes in blood pressure followed the experimental procedure.

¹ This investigation was supported (in part) by a research grant from the National Heart Institute of the Public Health Service.

DEMONSTRATIONS

(The numbers indicate the order of the demonstrations on the program of the meeting.)

D 42. A comparison of the growth and differentiation of the trigeminal ganglia with the ganglia of the cervical region in albino rat embryos. A. W. ANGULO, Division of Anatomy, Hahnemann Medical College.

It has been reported by some investigators that independent movements of the fore limb, elicited by means of strong stimuli, occur in the albino rat fetus prior to movements of the trunk induced by light tactile stimulation of the snout region.

A well graded series of albino rat embryos (silver impregnated) offered an opportunity to compare the sequential order of appearance of the above responses with the rate of growth and differentiation of the trigeminal ganglia and the cervical spinal ganglia. In 3.5 mm embryos (283 hours), the formation of neurofibrils in the trigeminal nerve is well advanced; no neurofibrils appear in the cervical ganglia. At 4.5 mm one can demonstrate the anlage of the three divisions of the trigeminal nerve; axon formation is beginning in the cervical region. At 6.0 mm the maxillary division of the trigeminal nerve forms a well defined trunk that can be demonstrated in gross specimens, and a subepithelial nervous plexus is present in the skin of this area; the nerve trunks destined to supply the fore limb can only be traced to the base of the limb. Fetuses of 13.0 mm show a subepithelial plexus in the skin of the fore limb; specialized nerve endings are observed in developing hair follicles of the snout region.

These findings indicate that trigeminal differentiation precedes that of the sensory-spinal system.

- D 9. Cultivation of rat liver tumors in fertile eggs.* Margaret I. ARMSTRONG, Alice E. GRAY and Arthur W. HAM, Department of Anatomy, University of Toronto.

In an attempt to obtain reasonably substantial amounts of homogeneous pure malignant tumor tissue of a kind permitting equally substantial amounts of normal functioning, regenerating, and embryonic tissue of the same variety to be obtained for control purposes, we have, by the method described by Taylor and Hungate for growing mouse mammary tumors in the yolk sacs of fertile eggs, injected fertile eggs with selected tissue from the livers of 41 rats fed a synthetic diet containing p-dimethylaminoazobenzene long enough for the livers to become enlarged and nodular. Microscopic studies showed tumors in 34 livers. Different types were seen: hepatomas, adenocarcinomas, papillary adenocarcinomas and a very anaplastic type we termed carcinoma simplex.

Those rat liver tumors that grew in yolk sacs were harvested and injected into further eggs. Five of the tumors thrived in this environment. One has now been transferred 43 times, another 31 times, and another 18 times. Two others gave every indication of growing in eggs just as well as the foregoing.

In our experience those rat liver tumors that lend themselves to successful serial cultivation in eggs are those which are malignant enough to have produced secondary growths in their host and which microscopically are of an anaplastic type. Of the three now being successfully serially transferred two are of the carcinoma simplex variety and the third is a papillary adenocarcinoma. Gross and microscopic preparations of these are shown.

- D 17. A note on the internal cremaster muscle.* William C. BARRETT, Department of Anatomy, Boston University.

The presence of an internal cremaster muscle in man, although mentioned in the older literature, is not generally recognized in current anatomical and histological texts. Investigation of testicular sheaths and spermatic cords from a series of individuals ranging from 3 to 70 years showed that smooth muscle is present in the internal spermatic fascia (tunica vaginalis communis) about the testis, but is not always present in the corresponding region of the spermatic cord.

Anterior to and on either side of the testis the smooth muscle bundles of the internal cremaster are arranged chiefly in two layers embedded in a loose connective tissue, the internal spermatic fascia. These layers, although discontinuous in places, are more definite and extensive than the layer formed by the skeletal muscle fibers of the external cremaster. Posteriorly the arrangement is less definite; and only isolated muscle bundles extend between the veins and coils of the epididymal duct on one side and the coils of the ductus deferens on the other. Superiorly, in the spermatic cord, a few bundles of the internal cremaster may continue upward between the veins of the pampiniform plexus in some individuals.

- D 31. The use of plastic embedding in the preparation of embryological material.* Alexander BARRY, Bradley M. PATTEN and Charles H. GLINES, Department of Anatomy, University of Michigan.

The obviously intriguing possibilities of the new plastics for embedding delicate materials in a condition suitable for teaching and research has led us, as well as a number of other groups, to work with them in several different ways. This demonstration is set up to show some of our current efforts to apply these methods to embryological material of various types. Most of the specimens demonstrated will be from a series on the skeletal development of human embryos. Other preparations will show the results with opaque specimens, dissections, and injections. Along with the more successful preparations other imperfect ones will be shown to illustrate some of the difficulties encountered. Mimeographed outlines of the methods in current use at Michigan will be available. It is hoped that others working in this field will take this opportunity to compare notes with us and offer their suggestions on some of the still troublesome phases of the techniques.

- D 15. Human anatomy in stereoscopic Kodachrome views.* David L. BASSETT, Department of Anatomy, Stanford University.

This demonstration consists of three-dimensional color photographs of anatomical dissections, bones and x-rays. It is illustrative of material in preparation for an atlas of human anatomy and is presented together with descriptions and line-drawings for the identification of structures.

- D 34. Polarized light detection of mineralized areas in undecalcified sections and in phosphatase positive tissues following incubation.* Leonard-F. BÉLANGER, Department of Histology and Embryology, University of Ottawa.

Celloidin embedded sections of mineralized material or of tissues submitted to glycerophosphate incubation, are mounted with a coverslip in 5% celloidin after alcohol dehydration. When thoroughly dry, the preparations are examined under the microscope between two pieces of Polaroid Glarefoil (JG30FAP). With polarizing axes parallel or crossed, considerable detail of the mineralized areas can be detected. Similarly mounted decalcified sections of the material must be studied as controls.

- D 45. Histochemical studies of membrane bone.* Gerrit BEVELANDER and P. L. JOHNSON, Department of Histology, New York University.

Microscopic slides and photographs will be shown of several developmental stages of membrane bone with special reference to the following: (1) Alkaline phosphatase; (2) glycogen; (3) mucopoly saccharide and (4) mineral salts.

Utilizing the Altmann-Gersh freeze-dry method of fixation, we have shown that alkaline phosphatase is present in osteogenic tissue and cells, in osteoblasts, osteoclasts and in the fibrous matrix of uncalcified bone. Glycogen and mucopoly saccharides are also present in osteogenic cells and fibers. In later development glycogen is present in both osteoblasts and osteocytes while the mucopoly saccharide appears throughout the matrix. As mineralization occurs there appears to be a disappearance of phosphatase in the matrix and a corresponding increase in the polysaccharide.

D 33. Comparison of various photographic emulsions for radioautographs by the coating method. R. BOGOROCH, Department of Anatomy, McGill University. (Introduced by C. P. Leblond)

In the past, this group has prepared "coated" radioautographs using a photographic emulsion of medium sensitivity, grain size, and resolution (Eastman Kodak Medium Lantern Slide Plate Emulsion). Recently, new emulsions have become available, some with a very high sensitivity and a low resolution such as Ansco Radioautographic Emulsion A, and some with a very low sensitivity and a high resolution such as British Kodak Autographic plates. Intermediate types of emulsion are Eastman Kodak NTB 2 and 3 and Ilford Half Tone Stripping film plates.

Coated radioautographs prepared with all these emulsions under comparable conditions will be demonstrated. The resolution, grain size, sensitivity, amount of fog, and ease of handling of the various emulsions will be compared.

D 43. The bicipital aponeurosis does not insert on the muscular fascia as claimed for it and for a number of other flat tendons, but inserts on the ulna. E. D. CONGDON and H. S. FISH, Department of Anatomy, The Chicago Medical School.

The bicipital aponeurosis like some other flat tendons has been generally said to blend with and therefore to insert on muscular (deep) fascia. The muscular fascia in this instance is the superficial antebrachial. By exposing the deep surface of the bicipital aponeurosis and related fascia the gross anatomy of the aponeurosis was made clear.

The aponeurosis which has about one fourth the cross section of the radial tendon quickly divides into (1), a strong oblique band which breaks up into collagenous fascicles inserting on the dorsal crest of the ulna, and (2), a thin distally directed sheet approximately two square inches in area whose fascicles blend with those of the superficial antebrachial muscular fascia distal to the band. The resulting fascicles which combine aponeurotic and fascial functions insert on the dorsal crest of the ulna.

The power arm for forearm flexion by the ulnar aponeurosis is about one and a half times as long as that for the radial tendon.

- D 49. Comparative studies of normal and avascular dog femur.*¹ Carl C. COOL-BAUGH, Department of Anatomy, Wayne University. (Introduced by F. Gaynor Evans)

The demonstration consists of selected stress-strain and density charts plus drawings illustrating the various surgical procedures used in the comparative studies made upon normal and avascular dog femurs.

¹ This research was supported (in part) by a research grant from the National Institutes of Health, U. S. Public Health Service.

- D 29. Fascial layers of the back.* George William COOPER, School of Medicine, Louisiana State University.

The larger fascial entities of the back have been studied by dissections and illustrated. Demonstration of some of the layers examined is to be made by artist drawings or photographs or both.

- D 18. Electron microscope studies on cells of the gastrointestinal tract and kidney.* Albert J. DALTON, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

Parietal cells of the stomach of the mouse will be shown to possess a filamentous "striated border" covering the free surface and lining the intracellular canaliculi. Other cell types possessing filamentous projections from their free surfaces, including chief cells of the stomach, intestine and gall bladder and the various parts of the nephron of the kidney will be demonstrated. Filamentous or thinly laminated material in the cytoplasm of exocrine cells of the pancreas similar in form to that seen in hepatic and hepatoma cells will be shown. The walls of the glomerulus of the kidney have been found to consist of a complete lining of endothelium resting on a basement membrane which in turn is covered by an incomplete layer formed by the fine processes of the cells of the visceral layer of Bowman's capsule. Details of the "myo-epithelioid" (polkissen) cells of the juxtaglomerular apparatus with very large cytoplasmic granules will also be shown.

- D 7. A method for hypophysectomy of rat fetus by decapitation.* L. V. DOMM and Pierre LEROY, Department of Anatomy and Walter G. Zoller Memorial Dental Clinic, The University of Chicago.

Pregnant female is etherized following which sodium nembutal is administered sparingly. A medial abdominal incision is made and a tepid, saline moistened, gauze is disposed around opening. Uterine horn is then gently pulled through incision and held in position by adjusting gauze. Head of embryo is located and held in place while a mild chromic catgut (eye suture) is inserted into antimesometrial wall in circumference around head and ends joined by a single loose tie. Within circle an incision is made sufficiently large to admit head. The latter is then expressed by gentle pressure whereupon catgut is tightened

in such manner as to keep shoulders and forefeet within uterus. A loop of surgical gut is tightened around neck and head severed above ligature. Chromic catgut is now inserted into each lip of uterine incision and by gentle upward pulls, exteriorized part of embryo is pushed back into uterus and ends tied. Uterine incision is then sutured. The catgut inserted into antimesometrial wall is now removed. Abdominal muscles and skin are sutured in customary manner.

It is advisable though unnecessary to keep uterus and embryo moistened with tepid saline. Rigid sterile precautions are unnecessary. No antispasmodic is used nor do embryos require an anesthetic.

Embryos of 16 days and older have been successfully decapitated. This operation does not impede body growth or muscular movements. Adrenal cortex atrophies. Results confirm observation of Wells.

*D 12. Comparative histology of lagomorph ovaries.*¹ Kenneth L. DUKE, Department of Anatomy, Duke University School of Medicine.

The mammalian order Lagomorpha lends itself admirably to comparative anatomical studies since (1) only two families are represented in the order, of which Ochotonidae has one genus (*Ochotona*, pika) and Leporidae has three (*Lepus*, hares; *Sylvilagus*, cotton tails; *Brachylagus*, pygmy rabbit), and (2) the two families vary in their habitat and anatomy. This difference is reflected in the ovarian histology. The ovaries of *Lepus* and *Sylvilagus* are similar histologically, while the ovary of *Ochotona* is different.

The ovaries of two species of *Lepus* (jack rabbit and domestic rabbit), one species of *Sylvilagus* (cotton tail), and one species of *Ochotona* (pika) are compared. *Lepus* and *Sylvilagus* have ovaries characterized by the presence of large masses of interstitial cells in the interfollicular stroma. These cells are not present in new-born females but appear after the second postnatal month of life. The interstitial cells arise chiefly from the hypertrophied cells of the theca interna of atretic follicles.

The ovaries of *Ochotona*, on the other hand, contain no interstitial cells in the adult: the stroma consisting of a fibrous tissue. The theca interna of atretic follicles does not thicken as it does in *Lepus*, nor do the individual cells hypertrophy to the same degree. However, in near-term fetuses and pre-pubertal specimens the ovaries of *Ochotona* have a stroma consisting primarily of large epithelioid cells.

¹ Aided by grant from Duke University Research Fund.

D 3. A silver technique for thick celloidin sections of the central nervous system. Mary L. DUNAWAY and James O. FOLEY, Department of Anatomy, Medical College of Alabama and School of Dentistry, University of Alabama.

The celloidin method is unsurpassed for neuroanatomical study of the brain and spinal cord. With the Nissl and Weigert type stains, a companion method, a silver technique, is needed.

Published silver techniques when used for 20–40 μ sections do not afford the necessary clarity. After experimentation with numerous silver baths, reducers, and counterstains the following method, which produces silver sections without the usual density, was devised. Fix tissue in 10% aqueous formalin. Cut celloidin sections 20–40 μ . Place sections in Davenport's alcoholic silver bath for 17–24 hours at 37°C. Rinse briefly in 95% alcohol and reduce in Pearson's silver-gelatin-hydroquinone reducer. After washing in water, if desired, tone with gold chloride.

After toning, sections may be counterstained. Best results are obtained by first staining with either carbol-thionin or with the periodic acid-Schiff technique of McManus, followed by treatment with phosphotungstic acid and staining in a mixture of fast green-Orange G.

A completed preparation displays the axons in black, the remnants of the myelin sheaths in orange, the connective tissue in green and the cell contents of the nerve body in blue, red, orange, or blue-green.

D 14. Electron microscope pictures of normal and poliomyelitis damaged ventral horn cells of monkeys. Donald DUNCAN, University of Texas Medical Branch. (Aided by funds from the National Foundation for Infantile Paralysis.)

No abstract.

D 19A. A mathematical approach to microscopic anatomy. Hans ELIAS, Department of Anatomy, Chicago Medical School.

The study of microscopic anatomy is generally carried out by observing infinitesimally thin slices of large, three dimensional organs. Many fallacies have occurred in the past due to misinterpretation of the flat microtome sections.

A geometrico-statistical method is presented which permits to draw conclusions concerning the three dimensional properties of organs and tissues, by measuring and counting parts seen in three single sections, cut perpendicularly to one another.

Visual evidence for the validity of the method is presented by means of models.

*D 48. Methods for studying the physical properties of bone.*¹ F. Gaynor EVANS and Milton LEBOW, Departments of Anatomy and Engineering Mechanics, Wayne University.

The tensile and shearing strength of bone samples of standardized size is determined by loading the sample to failure in a 500 pound capacity materials testing-machine reading to an accuracy of $\pm 0.5\%$. The deformation occurring in the sample is measured with a Porter-Lipp extensometer over a gage length of 1 inch. From the data obtained stress-strain curves, strength in lbs/in², per cent elongation and energy absorbed to failure can be determined. The deformations occurring in intact bones under static and dynamic loading are demonstrated with "stresscoat." The speed of transmission of a strain along

a bone is studied with SR4 strain gages and oscilloscopes. Density in bone samples is determined by use of a radio-active beta-ray emitter and Geiger counters. The fatigue strength of standardized bone samples is tested in fatigue testing machines. The above mentioned apparatus, as well as the tests themselves, will be demonstrated in Room 216 of the new Engineering Building at 623 Putnam.

¹ This research was supported (in part) by a research grant from the National Institute of Health, Public Health Service.

D 27. The prenatal development of the human femur. William J. FELTS, Department of Anatomy, University of Michigan. (Introduced by Wilfrid T. Dempster)

This demonstration presents the technique utilized, and some of the results obtained in a study of long bone development as exemplified by the prenatal human femur. Femora from specimens ranging from the ninth fetal week to term were stained with Alizarin Red S to demonstrate the progress of ossification, and then cleared by treatment with KOH and glycerine. By a special projection technique, line drawings of the frontal, medial, superior, and inferior aspects were made at constant magnification and measurements recorded. Some of the results of this, as yet incomplete, study are presented. By the method introduced by Julien Huxley (1934), coefficients of growth for various femoral characters and regions were determined. These are graphically presented. A major characteristic of the prenatal development of the femur is the high growth coefficient of its proximal and distal cartilaginous regions, and of the ends of the osseous diaphysis relative to that of over-all femoral length. A tentative interpretation of this phenomenon is offered. The changes in the amount of femoral torsion, of particular concern in the genesis of congenital dislocation of the hip are also shown. By the use of line drawings derived from roentgenographs, the state of development of the osseous trabecular structure is illustrated. The general course of femoral development is summarized by proportion drawings after the technique of Scammon.

D 38. Crossed renal ectopia, fused type. Carrie C. GILLASPY and O. J. VAN RENTERGHEM, Department of Anatomy, Des Moines Still College of Osteopathy and Surgery. (Introduced by Dr. Edgar Congdon)

A dissection of a white adult male, 64, disclosed a renal mass on the left side which extends from the top surface of the first lumbar vertebrae to the upper surface of the second sacral vertebrae. It measures 18.5 cm in its longest dimension by 9.5 cm at its widest point. The hili of the renal mass face anteriorly.

The left ureter enters the bladder normally. It follows the lateral margin of the renal mass. The inferior ureter of the ectopic kidney crosses its antero-medial surface, then crosses the mid-line at the level of the second sacral vertebrae and enters the bladder normally.

Three arteries arise from the aorta below the level of the inferior mesenteric artery. One enters the inferior hilus and two enter the lateral surface of the kidney mass. One artery arises from the aorta on a plane with the superior mesenteric artery and enters the superior hilus.

Two veins leave the renal mass to drain into inferior vena cava. The superior renal vein emerges from the superior hilus. Four tributaries to the superior renal vein are present. One emerges from the inferior hilus and two from the superior hilus. The remaining, left spermatic vein drains into the left renal vein. The inferior renal vein emerges from the inferior hilus. The right spermatic vein enters the right side of the inferior vena cava normally.

Two suprarenal glands are present. The right one did not wander across the mid-line with the displaced kidney. The left one is in normal position.

D 50. Skull fractures—types and mechanisms of production. E. S. GURDJIAN, J. E. WEBSTER and H. R. LISSNER, Wayne University College of Medicine and Grace Hospital.

This exhibit portrays the mechanism of production of linear, comminuted, depressed, and perforated skull fractures. The time of fracture production following impact is determined by means of electric strain gages cemented to cadaver heads. The relationship between energy, velocity, and type of skull fracture produced is portrayed. Generalized deformation of the skull following impact is also pictured. Fractures of the base of the skull are discussed. Experimental skull fractures of the human embalmed cadaver heads are compared with clinical examples of skull fracture.

D 44. A new staining method for the demonstration of the granules in the juxta-glomerular apparatus. The effect of nutritional deficiencies on the number of granular cells. Phyllis Merritt HARTROFT and W. Stanley HARTROFT, Banting and Best Department of Medical Research, University of Toronto.

By means of color photomicrographs and microslides, the appearance of granular cells in the glomerular afferent arterioles will be shown in sections of kidney, stained by a technique involving the use of Bowie's powder and Light Green, from normal rats and others suffering from certain nutritional deficiencies. (See abstract of paper 8 from platform.)

D 23. The extent of the descending root of the trigeminal nerve in the cervical region of the spinal cord in a human embryo of 8.5 weeks of menstrual age. Tryphena HUMPHREY, Department of Anatomy, University of Pittsburgh.

In a human embryo of 8.5 weeks of menstrual age (Homo 19, 26.5 mm C-R length), prepared by the pyridine silver method and serially sectioned in the transverse plane, both ophthalmic and maxillo-mandibular divisions of the descending root of V are readily distinguishable in upper cervical levels of the spinal cord. In transverse sections a slight indentation on the lateral surface of the descending

root marks the boundary between its ventrally situated ophthalmic division and its more dorsally located maxillo-mandibular subdivision, in which the mandibular fibers are most dorsal (Wallenberg, 1896).

Although fibers of all divisions of the trigeminal nerve extend well into the spinal cord in this human embryo, the maxillo-mandibular subdivision has approximately twice as great a caudal extent in the spinal cord as does the ophthalmic. The ophthalmic division extends through the upper third of C2 on the right and through its middle third on the left. The maxillo-mandibular portion, however, passes over a segment farther caudalward — throughout C3 on the right and even through the cephalic fifth of C4 on the left. The mandibular fibers extend farther caudalward than do the maxillary. The caudal extent of the maxillo-mandibular subdivision is an entire segment greater in this embryo than in one of 22 mm C-R length (8 weeks). (See abstract of paper 99 from platform.)

D 5. Lines of cleavage in the skin of the newborn infant. Cranford HUTCHINSON, Daniel Baugh Institute of Anatomy, The Jefferson Medical College.

The study of the lines of tension (Langer's lines) in the skin of newborn infants has been extended from the extremities (Anat. Rec., 103: 573, 1949) to include the thoracic, abdominal and perineal regions. The pattern of the lines in the infant when compared with that of the adult is not as strikingly different in the regions being demonstrated as it was in the case of the extremities but certain differences do consistently appear. The upper thoracic and gluteal regions are the most noticeable in this respect.

Incisions made in young children which follow carefully the lines of skin tension show but faint scars which practically disappear in subsequent years while the cosmetic effect of incisions made across these lines in early life sometimes becomes worse as growth proceeds.

Photographs and specimens will be demonstrated.

D 46. Some histochemical reactions of the testes of albino rats after intravenous injection of a nitrogen mustard, tris (2-chloroethyl) amine. James E. KINDRED, Anatomical Laboratories, University of Virginia.

This demonstration consists of sample sections from the testes of rats intravenously injected with sublethal amounts of tris (2-chloroethyl) amine. The material has been prepared to show the localization of alkaline phosphatase, carbohydrate, ribonucleic acid, and the reactions of dividing cells to protargol. Alkaline phosphatase present in the basement membranes of the seminiferous tubules and in the walls of the vasa is apparently not disturbed by the nitrogen mustard even though there is cellular destruction in the tubules. The pink reaction for carbohydrate as revealed by the periodic acid-Schiff reaction of MacManus and Cason, persisted in the poisoned rats in the tunica albuginea, basement membranes of the tubules, cytoplasm of the mast cells, outer membrane of the sperm heads, acrosome and acroblast of the spermatids and spermatid giant cells, cytoplasm of some of the interstitial cells, intertubular plasma, elastic and intermuscular fibers of arterioles, endothelium of the vasa, and faintly in the iodiosome of the spermocytes. In the methyl green-pyronin stain, the deep pink reaction (ribonucleic acid) of the

residual bodies cast off from the metamorphosing sperms persisted in the poisoned rats, but was abolished by incubation in 1.0% ribonuclease. In sections stained with protargol and fast green, the chromosomes of the spermocytes in division stained green and there was a blackening (argentophilic) reaction of the chromosomal fibers in contrast with faint or non-staining of the spindle fibers.

D 25. A slice reconstruction of the human hypothalamic nuclei. Wendell J. S. KRIEG, Department of Anatomy, Northwestern University Medical School.

The human hypothalamus has been modified in its shape by surrounding structures, with the result that the contained nuclei have been moulded and shifted, making them difficult to visualize without reconstruction. For the most part, the identification of nuclei agrees with those of Le Gros Clark and those established by a nomenclature committee in 1939.

The supraopticus diffuses is not here considered a separate entity, but the existence of a perifornicalis and a premammillaris as recognized by Clark is confirmed. The chief departures are (1) the separation of a filiformis from the dorso-medialis complex, with cells smaller and paler than those of the dorsomedialis; (2) the separation, based on cell types, of the lateral hypothalamic nucleus into anterior and posterior divisions; and (3) the recognition of a tuberomammillaris, characterized by large, dark, densely arranged cells of uniform type. This begins as several minute islets, further caudally it surrounds the nucleus tuberis, and when the latter is exhausted, expands and fills the ventrolateral sector of the hypothalamus. It conforms largely to the mamillo-infundibularis of Malone. Caudally, the nuclei posterior and dorsomedialis merge into the periaqueductal gray. The other nuclei of the caudal end of the hypothalamus come to a definite ending.

D 41. Growth stimulating effects of mouse sarcoma 37 on the sympathetic chromaffine complex of the chick embryo. Rita LEVI-MONTALCINI, Department of Zoology, Washington University.

Silver-impregnated sections are presented of the suprarenal glands of chick embryos carrying mouse sarcoma 37, and of normal control embryos. The tumor was transplanted in 3-day old embryos near the leg bud, or onto the yolk sac. The embryos were fixed between the 6th and the 15th day of incubation.

The sympathetic chromaffine complex of chick embryos carrying sarcoma 37 is highly hypertrophic and hyperplastic. An acceleration of the differentiation processes is also observed. Nerve fibers from the suprarenal visceral component invade the adjacent mesonephros.

D 32. Reconstruction of the heart of a human embryo of the ninth week. Richard LICATA, Department of Anatomy, University of Michigan Medical School.
(Introduced by Bradley M. Patten)

The model presented is one of a series of wax-plate reconstructions being prepared as part of an intensive restudy of human heart development. The model ($\times 100$) from Michigan Series EH377 (CR 31.5 mm), is dissectible to show the important internal structural relations. This stage of development is particularly

favorable for studying the early relations of septum spurium, the regression and the fusion of the base of the left venous valve with septum secundum, and the relations of septum primum and secundum in establishing the valvular complex at the foramen ovale. Certain features in the development of the sino-ventricular conduction system are shown, and especial attention has been given to working out the pattern of the developing coronary vessels. The branch of the right coronary artery which characteristically supplies the sino-atrial node is already established at this age. The semilunar valves have attained approximately their adult relations although they are relatively thick and still little differentiated histologically. Regression and retraction of muscle tissue from the ventricular faces of the developing tricuspid and mitral valves have commenced. The plug of plastic embryonic connective tissue of composite origin which closed the inter-ventricular foramen at the 17 mm stage (Kramer) remains massive, since the remoulding processes which underent the atrioventricular valves and thin the membranous portion of the interventricular septum have not yet become well advanced.

- D 39. *A study of lipid-laden "foamy" cells in the involuting thymus of the mouse.*
Lois A. LOEWENTHAL and Christianna SMITH, Department of Zoology,
Mount Holyoke College.

Lipid-laden cells, structurally similar, but cytochemically different from those described in certain lipidoses have been observed frequently associated with mast cells in the cortex of the involuting thymus of the mouse. Typical cells are large, polymorphic or rounded in shape and contain one or more usually eccentric nuclei. The cytoplasm is completely filled with droplets or vacuoles and granules; in many cells phagocytosed nuclear material is seen.

In frozen sections of thymuses treated with sudan black and other oil soluble dyes, the foamy cells were colored. In unstained frozen sections the cells were pale yellow. Baker's acid-hematein test for phospholipids was positive. Nile blue sulphate colored the cytoplasm blue. The Romieu and the Schultze variants of the Liebermann-Burchard test for cholesterol were negative. Certain of the cytoplasmic granules were anisotropic while the majority were isotropic. The cells exhibited a bright yellow fluorescence after short fixation in formol-calcium and freezing.

In paraffin sections the foamy cells were sudanophilic and acid fast. They were PAS positive and resisted digestion by saliva. When treated with ammoniacal silver, the granules were blackened. The cells were not metachromatic.

- D 11. *Extensive intratubular and extratubular edema of the rat testis compatible with spermatogenic function.*¹ Karl E. MASON, Samuel L. SHAVER, Harold C. HODGE and Elliott A. MAYNARD, Departments of Anatomy, and of Pharmacology and Toxicology (Atomic energy project), University of Rochester School of Medicine and Dentistry.

Rats were fed from weaning diets containing 2% uranyl nitrate hexahydrate and sacrificed at 60, 90, 120, 150 and 180 days. In 57 rats terminated at 150 and 180 days, 24% of testes showed varying degrees of gross enlargement and/or intratubular edema, 8% showed variable degrees of atrophy. The changes were

more frequently unilateral than bilateral. Enlarged testes, sometimes twice normal size and weight, were characterized by many seminiferous tubules with greatly dilated lumens and lined by a germinal epithelium only 2 to 4 cells thick but in active spermatogenic function. Progressive denudation of germinal epithelium was seen infrequently in these testes, but was common in the atrophic testes. Both states are attributed to progressive accumulation of intratubular fluid which, provided a certain pressure threshold is not attained, is compatible with spermatogenic functions.

In 9 other rats on stock diets given silver nitrate (0.15%) in the drinking water for 9 to 18 months, 5 showed marked bilateral enlargement of testes (2 to 3 times normal in weight) and were characterized by extensive intertubular accumulation of fluid, widely separating seminiferous tubules from the interstitial tissue and vascular supply; yet, with few exceptions, there were no morphologic or functional alterations in the germinal epithelium. Fluid accumulation was presumably gradual enough to avoid a pressure threshold sufficient to induce much tubular atrophy.

¹ Based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

D 30. Histochemical reactions of bovine spermatozoa. R. M. MELAMPY, J. C. PORTER and D. W. CHENG, Iowa State College. (Introduced by H. L. Foust)

Preparations will be demonstrated showing various reactions of bovine spermatozoa. Material stained with sudan black B, sudan IV, Nile blue sulphate, and oil red O indicate the presence of lipid material in the neck, middle piece, and tail. Baker's acid hematin method for phospholipid indicates relatively large amounts present in the posterior region of the head. Glycogen is not demonstrable by either the Bauer-Feulgen or periodic-Schiff reactions. The presence of desoxyribonucleic acid throughout the head may be shown by use of the Feulgen reaction. A gram-positive reaction is obtained throughout the sperm head. By applying Bodian's protargol method to bull sperm, it was observed that the anterior half stains gray whereas the posterior region appears quite dark. Basophilia is indicated in the head. Enzymes capable of dephosphorylating glucose-1-phosphate, fructose diphosphate, sodium glycerophosphate, yeast nucleic acid, and lecithin at pH 9 are present. The anterior half of the spermatozoon head stains more intensely than the posterior portion when prepared for alkaline phosphatase. A table indicating the chemical composition of bovine sperm will also be presented.

D 1. Nuclear morphology, according to sex, in nerve cells of several species and in various organs of the cat. Keith L. MOORE (by invitation), Margaret A. GRAHAM (by invitation) and Murray L. BARR, Department of Anatomy, University of Western Ontario.

Representative regions of the nervous system have been examined, in several mammalian species, to determine whether there is a sex difference in nuclear morphology similar to that described previously for the cat. In the dog, mink, marten, ferret, deer and goat, nerve cells of females contain a well developed intranuclear body which, however, is considerably smaller than the nucleolus.

A structure of comparable size is absent from nerve cells of males in these species. In the few rodents which have been studied, the morphology of the nucleolus-associated chromatin is complex and a sex difference has not been demonstrated.

The cells of various organs of the cat will also be demonstrated from this point of view.

*D 16. The blood vessels of the jejunum and ileum in man and certain laboratory animals.*¹ Rudolf J. NOER and John William DERR (introduced by Rudolf J. Noer), Departments of Anatomy and Surgery, Wayne University College of Medicine.

The blood vessels of the jejunum and ileum have been studied by injection techniques. Most of the specimens were prepared by injections of red and blue latex after which the specimens were cleared by a modification of the Spalteholz technique. Most of the intestinal loops have been imbedded in polystyrene for preservation and repeated study.

The following species have been studied: Man, chimpanzee, opossum, rabbit, wallaroo, hamster, dog, cat, rhesus monkey, raccoon, guinea pig, red fox, pig, marmot, sheep, goat and rat. Study of these specimens indicates that there are two general types of mesenteric patterns, one similar to that of man and the other resembling that seen in the dog. Some species have characteristics of their own and do not fall into either of the other groups.

The finer vessels in the wall of the intestine and the anastomoses between the mural vessels were demonstrated by India ink injections for comparison with the two-color latex technique and also to demonstrate finer vessels not filled by the coarser rubber.

¹ This work was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

D 37. Observations on formation of second polar body, pronuclei and first sedimentation spindle in fertilized rat ova. D. Louise ODOR and Richard J. BLANDAU, Department of Anatomy, University of Washington.

Studies on 1934 rat ova have made possible a more complete elucidation of the various changes occurring after fertilization than heretofore described. Detailed observations on the living ova were made with the phase microscope and were correlated with those on sectioned ova. Forty-seven percent of ova examined during the 1st hour, and 90% observed during the 3rd hour after ovulation were fertilized. The first alteration in the appearance of the fertilizing sperm head had occurred when the 2nd maturation division had reached the early telophase stage. By the time the 2nd polar body had been abstricted the entire sperm head had increased greatly in size and had lost much of its optical density. Various stages in the transformation of the enlarged sperm head into the relatively small male pronucleus; similar changes of the compact female chromosomal group into the female pronucleus; and subsequent pronuclear growth and migration will be demonstrated. Just prior to the formation of the 1st segmentation spindle all of the nucleoli of both pronuclei disappear. Actual fusion of the pronuclei has

not been observed. The chromosomes forming the metaphase plate of the 1st segmentation spindle were isolated from the oöplasm of living ova and photographed. Various stages of the 1st segmentation spindle, as well as the formation of the nuclei of the 2-cell egg, will be shown.

D 40. Speechmaster model for teaching articulation. Frances OLSON, Western Reserve University. (Introduced by C. C. Francis)

No abstract.

D 28. An atlas of the thalamus of the monkey for use with the stereotaxic instrument. Jerzy OLSZEWSKI, Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute.

This atlas consists of 22 photomicrographs of Nissl stained sections, and 12 photomicrographs of adjacent myelin stained sections of the thalamus of macaca mulatta. The magnification is 20 $\times$. The photomicrographs of Nissl stained sections are taken at intervals of 0.6 mm., those of myelin stained sections at intervals of 1.2 mm. The positions of the horizontal and lateral planes are indicated on the photomicrographs, and each plate is marked with the corresponding value of the frontal plane. The thalamic nuclei are delineated on the basis of their cytoarchitecture. The nomenclature is based for the most part on that used by Walker. In addition to the low power photomicrographs, a representative group of cells of each nucleus is reproduced under 200 $\times$ magnification, which shows the fine details of cell structure.

D 21. Dorsal trigeminothalamic tract in the brain stem of quadrupeds. James W. PAPEZ, Zoology Department, Cornell University.

The origin of the dorsal trigeminothalamic tract from the main sensory nucleus of the trigeminal nerve in the pons is shown in large photographs of a Weigert series of sheep brain stem. The tract takes its exit from the frontal end of the nucleus and inclines dorsomedially toward nucleus locus coeruleus, where it turns forward. In its course through the dorsal tegmentum of midbrain it is a large, well defined tract distinct from the rubroreticuloölivary pathway and other descending tracts in the tegmental region. Its termination in the dorsomedial surface of arcuate nucleus, in nucleus centrum medianum, and ahead in laminar nuclei is clearly seen. Marchi degenerations of this uncrossed tract in the cat have followed lesions of the main sensory nucleus of the trigeminus. This tract is a prominent structure in the dorsal tegmentum; and its origin and thalamic terminus are well shown in brain of horse and sheep.

*D 4. A modification of the Golgi technique.*¹ Anthony A. PEARSON, Department of Anatomy, University of Oregon Medical School.

This is a further modification of the Golgi method as recently outlined by Fox et al. (Anatomical Record v. 106, p. 303). Cerebral and cerebellar cortex, brain stem, and spinal cord can be prepared in the following manner:

Fix in 10% formalin. Material fixed for several months gave best results.

Place thin slices (2-3 mm. thick) of the central nervous system in 4 gm. of zinc carbonate, 4 gm. chromic acid and 2 gm. potassium dichromate, dissolved in 96 cc of distilled water plus 4 cc of formic acid for 3 days.

Remove, blot and place in a 0.5% silver nitrate solution for 3 to 6 days. Change this solution several times.

Dehydrate rapidly, clear in xylol, embed in low melting point paraffin and section in the manner outlined by Fox et al.

Sections are mounted and covered with a mounting medium. Plastic cover glasses are recommended.

¹ Aided by a grant from the U. S. Public Health Service.

D 22. Some examples of specific staining of non-nervous structures with silver.

Margaret M. POWERS, Grant L. RASMUSSEN and George CLARK, Department of Anatomy, School of Dentistry, University of Texas, Department of Anatomy, University of Buffalo School of Medicine and Department of Anatomy, Chicago Medical School.

In a survey of silver stains undertaken in an attempt to determine which was the most suitable for a projected study there were occasionally found examples of specific staining of non-nervous structures. Some of these were further studied in order to determine just how consistent results might be obtained. Among the variables studied were fixatives, decalcifying solutions and staining methods. Examples of the specific staining of the bony canaliculi, odontoblasts and Tome's processes, and of the dentinal tubules will be shown.

D 36. The application of phase microscopy to some problems in microscopic anatomy. Paul H. RALPH, Department of Anatomy, University of Washington.

No abstract.

D 2. The relationships of herniated intervertebral discs to the spinal cord and spinal nerves. Adrian F. REED, Department of Anatomy, Tulane University, School of Medicine and Homer D. KIRGIS, Section on Neurosurgery, Ochsner Clinic and Departments of Anatomy and Surgery, Tulane University School of Medicine.

Spines of cadavers have been cleaned, frozen with dry ice and sectioned longitudinally with a band saw. Specimens demonstrating degenerated or protruding intervertebral discs were embedded in plastic (Castolite). These preparations show a greater incidence of defective discs in the lower lumbar and cervical regions. It is apparent that intervertebral discs may protrude anteriorly or laterally as well as posteriorly. The relations of the spinal nerves and spinal cord to such protrusion, as well as the mechanism of compression of the former structures by herniated discs, are evident.

- D 20. *A wax-plate reconstruction of the nuclei of the dorsal thalamus of Macacus rhesus.* Glenn V. RUSSELL, Department of Zoology, Cornell University. (Introduced by James W. Papez)

A demonstration of a 4 × solid model of the dorsal thalamus of *Macacus rhesus*, and of an 8 × dissectible model of the principle nuclei of the dorsal thalamus of *Macacus rhesus* is presented. These models provide a precise three-dimensional representation of the shapes, sizes, and relative positions of the nuclei which make up this complex structure. The nuclei demonstrated in the dissectible model include the nucleus anterior, nucleus anterodorsalis, nucleus arcuatus, nucleus centralis lateralis, nucleus centralis medialis, nucleus centrum medianum, nucleus geniculatus lateralis, nucleus geniculatus medialis, nucleus lateralis anterior, nucleus lateralis dorsalis, nucleus lateralis posterior, nucleus limitans, nucleus medialis dorsalis, nucleus paracentralis, nucleus parafascicularis, nucleus pulvinar, nucleus suprageniculatus, nucleus ventralis anterior, nucleus ventralis posterior, and nucleus ventralis lateralis. The models are accompanied by a series of 9 labeled photographic views of the dissectable model.

- D 3. *Some effects of histamine.*¹ Carl J. SHEUSI, Department of Anatomy, University of Toronto. (Introduced by S. H. Bensley)

Since there is considerable evidence suggesting that histamine is concerned in anaphylactic reactions, hyperimmunity and, hence, perhaps in collagen diseases, histological studies of the reaction of tissues to one injection of histamine were begun.

To date, 9 male albino mice have been injected subcutaneously in the midline of the back with 0.1 cc of 0.02% aqueous solution of histamine acid diphosphate. One animal of the series was sacrificed immediately after injection. The remaining animals were sacrificed at intervals ranging from 24 hours to 8 days after injection.

Histological studies indicated that there were progressive changes in the skin, spleen, adrenal and testes. The changes to be demonstrated include: (1) an increase in the number of fibroblasts, acidophilic leukocytes and mast cells in the skin at the site of injection; (2) an increase in mast cells and megakaryocytes in the spleen; (3) an increase in the number and size of certain cells in the adrenal which have the same staining reactions as the interstitial cells of the testis; (4) an increase in the number and size of the interstitial cells of the testis.

These changes apparently reach the maximum around the fourth or fifth day following one injection of histamine.

¹ Aided by a grant from the Canadian Arthritis and Rheumatism Society.

- D 35. *Histochemical studies in tissue culture.* Norma SHIFRIN, Detroit Institute of Cancer Research. (Introduced by William L. Simpson)

Histochemical studies have been made of the distribution of carbonyl groups in mouse liver *in vivo* and *in vitro*.

Carbonyl groups were stained in neutral formalin-fixed tissue with 2,4-dinitrophenylhydrazine (DNPH), and in fresh tissue with fuchsin sulfurous acid (FSA). Hanging drop cultures of liver from day old ND strain mice were stained *in toto* after one to seven days incubation. Frozen sections of livers from ND mice of the same age as the cultures were stained for comparison. Non-sublimate-treated materials were used as controls on the FSA reactions.

In vivo liver, although negative to FSA, showed occasional extracellular globules and very fine cytoplasmic granules following DNPH staining. In most fibroblastic and epithelial cells of cultures, both stains showed many large positive granules which became progressively larger and more numerous with increasing age of the cultures. Older cultures showed some positively stained material in the FSA controls, possibly due to the liberation of aldehydes as acids accumulated. More stained granules always appeared in sublimate-treated cultures.

In size and position, granules stained by DNPH and FSA resemble the refractile, Sudan-positive granules seen in ageing cultures. The FSA experiments indicate that these globules contain a lipidic carbonyl component, possibly derived from acetal phosphatide, plasmalogen. The absence of this compound in non-cultured liver, suggests a difference in fat metabolism of liver cells *in vivo* and *in vitro*.

D 10. The persistence of argentaffin cells in the excised intestine of the dog and rabbit. W. T. SOHN and L. B. AREY, Department of Anatomy, Northwestern University Medical School.

Contrary to the usual opinion that argentaffin cells are extremely labile, it can be shown that they persist in the intestine for an astonishing length of time. In the duodenum and sigmoid of the rabbit and in the duodenum and rectum of the dog these cells of the mucosa remain essentially normal in total appearance and in their granular arrangement, after staining with a modified Bodian's protargol method, for about 24 hours. Between 48 and 96 hours there is deterioration until not more than one-half of all the argentaffin cells still appear essentially normal. Between 96 and 168 hours there is further deterioration and, although perhaps one-third are then undemonstrable, the remainder still stain even though they are more or less atypical. The persistence was better in the duodenum of the dog than in the rabbit; in the sigmoid-rectum region the reverse was true. In view of the prompt degeneration of the mucosa as a whole, the long continued persistence of recognizable argentaffin cells is remarkable.

D 26. Demonstration of normal and altered nerve terminals in bulbar nuclei, as stained by an intensified protargol method. W. A. STOTLER, Department of Anatomy, University of Oregon Medical School.

Preparations and photomicrographs of nuclei of the acoustic system and adjacent areas. (See abstract of papers presented by title and from platform.)

D 6. *A modified freeze-dry apparatus.*¹ Norman M. SULKIN, Department of Anatomy, St. Louis University.

A glass freeze-dry apparatus has been designed which is especially applicable for use in histochemistry. Temperature of the tissue is controlled by means of thermocouples so that the optimum rate of diffusion can be obtained. Water vapor is trapped by a system of baffles close to the tissue, such as described by Stowell. A high vacuum is maintained with the use of a specially designed mercury diffusion pump and a mechanical fore-pump. The pressure of the vacuum is measured directly by means of a McLeod Gauge. The tissues are infiltrated with paraffin during evacuation so that they are subjected to high temperatures for only a few minutes or less. The heat to melt the paraffin is produced by a heating element within the tissue holder and is regulated by a Rheostat-potentiometer. The apparatus takes up very little space and is inexpensive to build.

¹This investigation was supported by a research grant from the National Heart Institute, U. S. Public Health Service.

D 24. *Observations on the extension of nerve fibers into the odontoblastic matrix.* Ira R. TELFORD and Margaret M. POWERS, Department of Anatomy, University of Texas School of Dentistry.

The innervation of the dentin is still a controversial subject due primarily to difficulties in the differential staining of the delicate nerve fibers. With modifications of several acceptable neurological procedures these nervous elements can be traced in human teeth from the pulp to the odontoblastic layer and finally into the predentin region. In rat molars the nerve fibers can be traced well into the dentin proper.

D 19. *Microscopic observations of the settling of masses of moving sludged blood to the bottom of blood vessels and the formation of venous thrombi in living animals.* Louise WARNER, Hull Laboratory of Anatomy, University of Chicago. (Present address, National Naval Medical Center, Naval Medical Research Institute, Bethesda, Maryland.) (Introduced by Melvin H. Knisely)

The settling of agglutinated blood cells during life was partly described in *Science*, November 7, 1947, and parts of the process demonstrated by motion pictures taken through a horizontal microscope (*Anat. Rec.*, 100: 784, 1948).

Continued study has shown that blood cell masses settle out under partly definable physiological and physical conditions.

When settled blood cells are stationary they transport no oxygen; settling thus contributes to tissue anoxia.

After settling, masses (1) may or may not cement together forming a thrombus and (2) may or may not cement to the bottom of the vessel.

For details see Warner, L. Thesis, University of Chicago, 1949. For thesis summary see Collens, W. S. and Wilensky, N. D. "Peripheral Vascular Diseases" Springfield, C. C. Thomas, 1951, Chapter on Thromboembolic Diseases.

This demonstration consists of forty still photographs showing the method of study, the results of one experiment, and some accessory studies, prepared by LIFE magazine, photographs by F. W. Goro.

D 13. Electron micrographs showing the submicroscopic network of fibrils in the groundsubstance of connective tissue. F. WASSERMANN, Division of Biological and Medical Research, Argonne National Laboratory.

Small pieces of natural membranes of subcutaneous connective tissue of mouse, rat, and guinea pig were re-spread on the slide. This technique permitted study of the intact connective tissue with the electron microscope. The electron micrographs (E.M.G.'s) show a network of solitary fibrils as a regular component of the ground substance. This is seen in unfixed, air-dried specimens and is preserved by fixation with neutral formalin. Acid-containing fixing agents dissolve the fibrils. By comparing the light-photomicrograph of the silver-impregnated tissue with E.M.G.'s at 3000 to 4000 $\times$ one finds the pattern of the argentophilic fibers repeated at the lower scale. While the diameters of the finest fibers visible in the light microscope are of the order of about 2000 Å, the widths of the fibrils vary from about 300 to 800 Å. The cross-banding along the axis of the fibrils shows a periodicity ranging from 300 to 800 Å. Values smaller than the average periodicity of collagen are prevalent. Where solitary fibrils unite the bands of the same density correspond at the same level. Fibers of diameters small enough to be contained in the electron microscopic field are composed of fibrils that are identical with the solitary fibrils. Besides the fibrils still finer threads (protofibrils) are regularly present in the ground substance.

D 47. The visualization of capillary beds by intravascular precipitation of lead chromate. T. Walley WILLIAMS, Department of Anatomy, School of Medicine, West Virginia University.

The vascular beds of several different mammalian tissues and organs are demonstrated, prepared by a technic previously published in the ANATOMICAL RECORD, V. 100, No. 1, January 1948. The method is based upon the precipitation of lead chromate within the capillaries and larger vessels, in contrast to older methods of injecting previously formed masses into the vascular system. We believe this method to be a distinct improvement in several respects. Injection is followed by dehydration and clearing of the soft tissues. Large blocks of the treated tissues are embedded in polished plastic mounts for examination under the wide-field, binocular microscope. The tissue, however, may be stored permanently in methyl salicylate; after removal from this oil it may be mounted as above or, after appropriate treatment, placed in paraffin blocks for routine sections of various thicknesses which may be stained or mounted unstained. This method was developed as part of some work, now in progress, on the capillary beds of certain mammalian organs. Modifications and additions to the technic will be ready for publication in the near future.

MOTION PICTURE DEMONSTRATIONS

- Dm 11. Mitotic changes accompanying continuous irradiation.* Austin M. BRUES and (by invitation) Agnes N. STROUD and George SVIHLA. Division of Biological and Medical Research, Argonne National Laboratory.

The mitotic figures observed in tissue cultures of chick embryo muscle show great variation in duration and behavior. Under similar conditions of irradiation by tritium oxide at dosage rates of 62.5 to 167 rep/hour, certain mitoses appear entirely normal. Others show evidence of injury to the spindle system, with delayed division accompanied by lack of chromosomal orientation, rotation or shifting of a central mass of chromosomes, as well as chromosome clumping. Cell division may become dissociated from the mitotic cycle, resulting in pinching off of two daughter cells with unequal numbers of chromosomes or pseudo-amitotic figures.

- Dm 10. Cell structure — a comparative study of the cytological characteristics of normal and malignant cells with phase and electron microscopy.* G. O. GEY and F. B. BANG, Departments of Surgery and Medicine, The Johns Hopkins University School of Medicine and Hospital.

The remarkable properties of the protoplasm of living cells must be related to the basic fibrous gel structure and the character of their specific cellular components. Phase optics and time-lapse photomicrography allow a remarkable comparison of the living cell's composition and structure with similar but fixed structures, in the same or an homologous cell, when studied under the electron microscope. Emphasis will be placed on the ability of cells to produce membranous and filamentous processes and to ingest and discharge particles. The relation of the delicate filamentous processes and other fibrous components to the hyaloplasm and the character of the intracellular traffic will be shown. Some of the differences between specific rat malignant cells (T-333) produced in continuous cultures and their normal prototypes (14p), as determined by this study, will be pointed out and compared with other continuously cultured cell types.

- Dm 5. Intrinsic contractility in the embryonic rat heart.*¹ E. K. HALL, Department of Anatomy, University of Louisville School of Medicine.

The hearts of 203 rat embryos in the 10, 11, 12 and 13 day stage were studied. The embryos were placed in warm (30–32°) Ringer, and the heart rate determined (average = 91.3/minute). The heart was then excised and the rate again determined (average = 106.9/minute). The heart was then divided between atrium and ventricle. The atrial fragments beat almost exactly (1.94) twice as fast as the ventricular fragments (119.0 vs. 61.3/minute). The dominance of the sino-atrial contractions may be inferred.

Incompletely sectioning the heart leaves a narrow strand of tissue between atrial and ventricular fragments; such strands are sufficient to synchronize the contractions of the heart. Partial heart block may occur at the sino-atrial or the atrio-ventricular junction; such hearts may return to normal function. Some heart fragments continue to beat after 24 hours in Ringer, and a few after 48

hours; in these fragments, contractions may be restricted to a small region, while the rest of the fragment is degenerating. Subdivision of the ventricular fragment into as many as 4 pieces does not accelerate the intrinsically slow rate of the ventricular tissue.

Silver impregnations (deCastro) show that the first nerve fibers invade the heart only at the 14 day stage. The inception of the beat and its transmission must therefore be referred entirely to the myocardium.

¹Supported by a grant-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

Dm 8. White thrombo-embolism in the hamster cheek pouch after trauma, infection and malignant neoplasia. Brenton R. LUTZ, George P. FULTON, and Robert P. AKERS. Department of Biology, Boston University. (Introduced by William C. Barrett)

A method of transilluminating the hamster cheek pouch for cinephotomicroscopy of the small blood vessels at magnifications of 200 to 1200 $\times$ is shown. The structural components of the arterioles, capillaries and venules, as well as the conditions of flow of the formed elements are shown in the normal animal. The smooth endothelial wall, perivascular mast cells and adventitial cells are seen at the highest magnification. Endothelial trauma produced by occlusion with artery forceps or by strong stimulation by a microelectrode produced platelet thrombi, fragmenting into emboli. Mixed platelet and leukocytic coatings and emboli were found extensively during generalized *S. Aureus* infection, intra peritoneally induced. Methylcholanthrene-induced malignant sarcoma, transplanted into the cheek pouch, produced platelet emboli and mixed platelet and leukocytic coatings, as well as leukocytosis, as observed in the small vessels of the opposite cheek pouch. Emboli, however produced, sometimes plugged small arteries and capillaries. Sludged blood was not observed during any of the experimental procedures.

*Dm 1. A moving picture demonstration of the effects of elevated and reduced temperature upon the resistance of guinea pigs to asphyxia.*¹ James A. MILLER, Jr., Faith S. MILLER, Carolyn B. FARRAR, Department of Anatomy, Emory University School of Dentistry.

This Kodachrome moving picture demonstrates that reduction of colonic temperature increases the asphyxial survival of neonatal guinea pigs by 50% per 10°C. The film shows also that although less effective than in neonatal animals, temperature reduction produces a similar straight line increase in asphyxial survival in young adult guinea pigs. Likewise, experiments are illustrated showing that cooled animals can completely recover from exposures to 95% N₂ + 5% CO₂ which are lethal to warmer animals. These experiments agree with earlier findings (Miller '49a, '49b) and cast further doubt upon the advisability of warming infants suffering from asphyxia neonatorum.

¹Supported in part by a grant from the National Institutes of Health.

Dm 12. Blood and marrow cells with phase cinematography. Paul H. RALPH, Department of Anatomy, University of Washington.

No abstract.

Dm 6. The anatomy of joint action: I. Hand and wrist. Irving REHMAN, and George D. MASON, Department of Anatomy, School of Medicine, University of Southern California.

A teaching film to illustrate the functional anatomy of the hand and wrist as seen in live action, X-ray motion pictures and anatomical dissections.

Dm 2. Motions of the seminiferous tubules of rat and dog. Edward C. ROOSEN-RUNGE, Department of Anatomy, University of Louisville.

The motion picture demonstrates that seminiferous tubules, when isolated and freely suspended in Ringer's solution at about 36°C., are capable of motions which affect their shape and the width of their lumen. The motions have been observed in the intact testis but it has been impossible so far to photograph them in situ. The motions are slow and undulating and show a vague periodicity of 40-60 seconds. The vigor of the movements varies greatly in different tubules. It appears that relatively short segments of a tubular wall contract slowly at intervals, the contraction traveling a varying, but usually short, distance along the tubule. It has not been determined whether the direction of travel is constant. No motion was observed in tubules from immature testes.

In all probability the movements are caused by contraction and relaxation of the Sertoli cells. This may be inferred from the fact that the tubules contain no other element which appears capable of contracting. Attention is called to the fibrillae which have been described in the Sertoli cells of most mammals. In the dog these fibrillae can be demonstrated with particular ease. The great variability of the movements may, perhaps, be explained by the different structure of the Sertoli cell complex in different phases of the spermatogenic cycle.

Dm 3. A comparison of inert and active foreign body implants in tadpoles as recorded by ciné-photomicrography. Carl Caskey SPEIDEL, School of Anatomy, University of Virginia.¹

These motion pictures made directly from living tadpoles portray the typical tissue reactions to implants of chemically inert and active substances. As an example of the former, a case history of an implant of human hair is presented. The scenes illustrate the principal microscopic features for the following times after operation: the early changes after 10 minutes, 5 hours, 1 to 4 days; the later changes at intervals of from 5 to 45 days. A sharp contrast is afforded by case histories of implants of coarse magnesium powder. Magnesium causes progressive irritation of the tissues as it gives rise to magnesium hydroxide and hydrogen. Selected case histories show the changes over periods of from 10 minutes to several months. These include the initial formation of gas bubbles, the mobilization of leukocytes, the process of encapsulation, and the induction

of large vesicles. The increased use of magnesium in modern warfare has resulted in its frequent occurrence in war wounds; these motion pictures, therefore, have a practical bearing (cf. paper no. 249).

¹ Aided by a grant from the American Cancer Society (Committee on Growth).

*Dm 4. A color motion picture showing factors controlling lens regeneration from cells of the dorsal iris in salamander eyes.*¹ L. S. STONE, Department of Anatomy and Osborn Zoological Laboratory, Yale University.

The release of lens regeneration from dorsal iris tissue in the adult eyes of *Triturus v. vididescens* is shown by removal of the normal lens, by isolation of the dorsal iris into a separate chamber, and in many types of iris grafts. Conditions are also demonstrated under which multiple lenses are developed simultaneously. The inhibition of lens regeneration is shown by the presence of transplanted normal lenses of the same or different species, by the presence of lenses in certain stages of regeneration, by some carcinogens and by injections of aqueous humor from eyes containing lenses.

¹ Aided by grants from the U. S. Public Health Service and the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

Dm 7. Persistence of active vasomotion along blood and lymphatic vessels in the bat's wing after denervation. R. L. WEBB and P. A. NICOLL, Departments of Anatomy and Physiology, Indiana University School of Medicine.

Observations reported previously indicate that active vasomotion exhibited by terminal arterioles, veins, and lymphatics is not regulated to any great extent by the nervous system. This is in contrast to vasomotor responses of larger arteries which are primarily under nervous control.

The motor responses of these various blood and lymphatic vessels are shown here in both normal wings and those with their nerves sectioned and degenerated.

*Dm 9. The circulation in living autografts of spleen.*¹ R. G. WILLIAMS, Department of Anatomy, School of Medicine, University of Pennsylvania.

Autografts of spleen were made into tantalum and mica chambers installed in rabbits' ears and studied repeatedly with the compound microscope for many months. Grafts of red pulp reconstituted themselves as complete lobules with central penicillus arteries surrounded by Malpighian bodies. The latter were surrounded by sinusoidal regions into which many arterioles discharged through "ampullae of Thoma." There was no typical capillary plexus in the sinusoidal region. In the latter some vascular channels were lined with cells having unusually prominent nuclei but others had no continuous lining that could be detected. There was no active mechanism for controlling the flow in sinusoids.

Blood storage in sinusoids seemed to be dependent upon the size of openings into and out of them. This was determined by increase and decrease in the cell columns surrounding the channels. Protoplasmic masses underwent intravascular fragmentation in a manner suggesting platelet formation. Intravascular phagocytosis of particulate matter occurred. Phagocytosis of red cells was rare. Lysis of stationary red cells in sinusoids was not observed. Red cells in sinusoids could be evacuated by external pressure slowly applied but not by pressure acutely applied or by administration of adrenalin. In some respects the grafts seemed to be in the blood stream rather than the reverse.

¹ Aided by a grant from the American Cancer Society.

